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# GENERALIZED MEGALOCARYOCYTIC REACTION TO SAPONIN POISONING

By J. Firket and E. S. Campos

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Our interest in the subject of this investigation was aroused by the occurrence of a peculiar case of extreme anæmia in which there was complete aplasia of the bone marrow together with a new formation, in the liver and spleen, of myeloid tissue in which megalocaryocytes predominated. This case, in itself worthy of publication, we could not explain on the basis of anything that could be learned of the man's history, but in attempting to imitate it by any means in our power, we found that a very similar condition could be produced by saponin poisoning. Bunting, as we found later, had shown that saponin poisoning destroys many of the blood platelets and produces a multiplication in the bone marrow of the megalocaryocytes; these observations we have been able to confirm.

The case of anæmia was as follows: *Clinical History:* J. W., a healthy man, aged 40 years, began to feel weak during December, 1920. He continued working until January 11th, 1921, but with increasing fatigue, weakness, dyspnœa and pain in the lumbar region. At about that time he developed œdema of the ankles and puffiness of the face. On January 13th the blood examination showed 1,744,000 R. B. C., 5,300 W. B. C., and 25% hæmoglobin. Two days later, during which he had been semicomatose, these figures were, 1,272,000 R. B. C., 4,200 W. B. C., and 17% hæmoglobin. The color index was 0.71. The blood picture showed especially anisocytosis, poikilocytosis, 48% neutrophilic polymorphonuclear leucocytes, 2.5% myelocytes and a very few normoblasts. Platelets were practically absent. Hæmolysis began at .48% sodium chloride and was complete at .32% sodium chloride. There were no other positive symptoms. Notwithstanding two transfusions of 500 c.c. each of citrated blood, which raised the red-cell count to 2,000,000 with 34% of hæmoglobin and 3,360 white cells, the patient

sank deeper into coma, the pulse became weaker and death followed on January 20th. Autopsy ten hours after death by Dr. Athens.

*Anatomical diagnosis:* Extreme anæmia, aplastic bone marrow; splenic tumor; focal necrosis of liver and spleen; extensive myeloid changes in liver and spleen; acute sero-fibrinous pericarditis and pleurisy; tuberculosis of mediastinal glands; caseous encapsulated nodule in left lung; catarrhal bronchitis.

Deposits of pigment were found in the liver cells, a slight hæmorrhage in the fundus of the eye and a complete aplasia of the marrow of the long bones, which was entirely fatty. Throughout the red pulp of the spleen and between the hepatic cells myeloid elements were present. It is very striking that, although in this myeloid tissue one finds normoblasts and myelocytes in fairly large abundance, proportionally the most numerous elements are megalocaryocytes. In other words, the number of megalocaryocytes is relatively larger than in any other myeloid tissue: this is especially in contrast with what is seen in other myeloid metaplasias of spleen and liver, as, for example, in cases of myeloid leukæmia. Some of these megalocaryocytes show a large, swollen, clear nucleus, with very little chromatin; others have a shrunken, deeply stained, almost pyknotic nucleus.

In the spleen there were some megalocaryocytes which had gone through the capillary system of the liver into the pulmonary circulation, where they were found in the sections of the lung.

As far as the diagnosis is concerned, the myeloid, or better, the megalocaryocytic reaction of the spleen and liver, the presence of focal necroses in the liver and spleen, the presence of iron pigmentation in the tissues and the absence of marked hæmorrhages, lead us to abandon the diagnosis of pure aplastic anæmia.

It seems probable that there was a poisoning of some sort and indeed the history records the fact that the patient was exposed four years before (but not since then) for about a year to benzol vapors and that the room in which he had worked lately was filled with fumes from soldering. It is obvious that the late effects of exposure to benzol vapor must be considered in our effort to explain the existence of complete aplasia of the bone marrow with the presence of myeloid tissue in other organs. Selling found that myeloid metaplasia occurred in the spleen after the cessation of the myelotoxic action of the poison and at a time when the aplastic bone marrow was regenerating itself. It was not possible to enter upon experiments to decide whether a stage in the recovery from benzol poisoning might be reached at which myeloid metaplasia of distant organs could coexist with a still completely aplastic bone marrow. This, as well as the question of the possible production of abundant megalocaryocytes in distant organs, remains to be determined. Our interest took a different turn toward an effort to find some poison which would produce this condition.

Saponin is well known as a powerful hæmolytic agent, producing, as Foa states, a severe anæmia with complete aplasia of the bone marrow and simultaneously a myeloid metaplasia of the spleen. This then seemed the most suitable substance for our experiments.

Chemically the term saponin is applied to a number of glucosides which, according to Cushny, have many features in common both in their chemical properties and their pharmacological action. While the saponin bodies seem to have a localized destructive action upon the cells with which they come in contact, their effect is practically restricted to the blood and hæmopoietic organs when injected into the body.

Saponin had been used in the experimental production of anæmia in rabbits by Bunting in 1906. Later, as mentioned, in his paper of 1909 he records the fact that saponin destroys blood platelets as well as red corpuscles and stirs up in the bone marrow an excess production of megalocaryocytes. Isaac and Möckel in 1910 state that saponin destroys the bone marrow, leaving it aplastic and producing a myeloid metaplasia of the spleen resembling the condition to be found in the hypothetic "acute aleukæmic myeloid leukæmia."

The results of these experimenters led us to believe that by using saponin we might produce in rabbits a condition resembling that described in the fatal case in the human being.

The first experiments showed a conspicuous new formation of myeloid tissue in the liver and spleen with one feature which has not been observed by the other investigators, namely, the predominant new formation of megalocaryocytes in those organs. This becomes the chief point in our results, because on account of their function in forming blood platelets these cells assume a great importance in connection with the destructive action of saponin upon the platelets. Bunting had mentioned this rather specialized destructive action and we proceeded to study it in further detail and to consider the subsequent regenerative processes.

Before relating our experiments and observations, it is a pleasure to acknowledge our indebtedness for the opportunity for work afforded us in the laboratory of Dr. W. G. MacCallum.

## Material and Methods

*Saponin:* The sample used was the same for all the experiments. It came from The Heyl Laboratories, Inc., Chicago. All the doses were weighed with the same balance and, on account of the instability of solutions of this glucoside, fresh saponin was dissolved for every experiment.

The physiological salt solution, used as solvent, was also made up with the same sample of chemically pure sodium chloride (Merck) previously dehydrated by one

hour's exposure at 150° C. The solution used was always at the strength of 0.9%.

All the animals used were of the same variety of Belgian hare, and none of them were very young. They were all submitted to the same conditions of food (wheat, water and occasionally fresh lettuce) and kept in the same room at a fairly constant temperature.

Each of the forty-five rabbits was given a number as soon as it was used once and this number was not changed afterwards.

All the injections were made intravenously after shaving and cleaning the region of the marginal vein of the ear. As the localized necrotic action of saponin, when used in relatively strong concentrations, was very marked, there usually occurred thrombosis of the veins of the ear with very often œdema, and later on, fibrosis of the cellular tissue surrounding the vein. This, in a few instances of long repeated injections obliged us to inject into the veins of the abdominal wall, without any other trouble than the same localized action just mentioned.

*Autopsies.* Each dead rabbit was submitted to autopsy. These were made most frequently immediately after death, as the rabbits were usually killed. Some rabbits found dead in their cages the next morning were examined as soon as possible, so that the tissues were not in any case exposed to post-mortem changes for a period of more than ten hours. All autopsies were performed according to the same method. The liver, spleen, bone marrow of the femur, tibia and humerus, adrenals, lungs and kidney were fixed. In many autopsies the lymph glands were also preserved.

Zenker's formol fluid (Helly's formula) was used as fixing agent; occasionally 10% formalin was used.

The organs, embedded in paraffin, were then sectioned, the sections being from 5 to 7.5 in thickness.

Longitudinal sections of the whole organs, as far as possible, and transverse sections of the bone marrow of each specimen, were made. This is important, as only a thorough examination of the marrow of all the long bones can give a true impression of the condition of this hemopoietic apparatus. A longitudinal section of the complete spleen was also cut in most cases.

The staining methods used were: Hæmatoxylin (Grübler's) and eosin; Wright's stain and the Poulenc "Panchrome de Laveran."

For blood examination and blood counting the usual routine methods were employed. The same pipette and counting-chamber (Thoma-Zeiss) were used for all the counts of one animal. The diluting fluid for red corpuscles was 0.9% salt solution and the special fluid used for platelets (see below); for the white cells, Türck's fluid. As soon as the sample of blood was diluted, the pipette was shaken in an electric shaker for from five to ten minutes to avoid any agglutination and precipitation.

The hæmoglobin estimation was made always with the same Gowers-Sahli hæmoglobinometer.

In order to make the report of our results clear, we shall proceed in the following manner: the experiments will be selected according to the problem to which they are related; by that means we shall deal first with the question of blood destruction which has led us to study (1) the resistance of the red cells to saponin; (2) the specific action of saponin on blood platelets *in vivo* and *in vitro;* after this the process of regeneration will be described and with it the most appropriate protocols of experiments and autopsy records.

Finally, we shall have to consider the question of the mechanism which leads to the so-called "aplasia" of the bone marrow after injections of saponin.

### Studies of the Resistance of Red Cells to Saponin

When we began systematically our study of the action of saponin, we realized that in order to come to an understanding of the blood curve, we had to study not only the process of regeneration but also the process of destruction. Indeed, there was a possibility that the resistance of the erythrocytes of normal rabbits might change after saponin injection, making difficult our understanding of the blood curve.

Realizing this, in a first series of experiments we investigated the resistance of the red blood cells of the normal rabbits to our sample of saponin. After we had found this we tested the resistance of the red blood cells of the same rabbits after they had been injected with saponin.

In a third series of experiments, we investigated the same property of the blood in splenectomised rabbits. This was done, because it had been shown by Pearce and others, in their experiments on splenectomised dogs, that there is, after splenectomy, an increased resistance of the red cells to hypotonic salt-solutions, hæmolytic serum, saponin, and other injurious substances.

*Technique.*—Only washed blood-cells and not the blood *in toto* were used for the hemolytic test. The blood was taken through a puncture of the marginal vein of the ear and allowed to drop freely into a 1 per cent solution of sodium citrate. The suspension was then centrifuged, the plasma removed by means of a pipette, and the red corpuscles washed three times with a 0.9% NaCl solution. A suspension of washed red cells of about 3% was then made in the same physiological solution.

Saponin was prepared in the same salt solution at the strength of .25 mmgr. saponin for 1 cc. of physiological salt solution. This mother solution was diluted with physiological salt solution as follows:

| Tube No. | Sol. of Saponin .25 mmgr. for 1 cc. salt sol. .9% | 0.9% Salt solution |
|---|---|---|
| 10 | .10 cc. | .90 cc. |
| 11 | .09 cc. | .91 cc. |
| 12 | .08 cc. | .92 cc. |
| 13 | .07 cc. | .93 cc. |
| 14 | .06 cc. | .94 cc. |
| 15 | .05 cc. | .95 cc. |
| 16 | .04 cc. | .96 cc. |
| 17 | .03 cc. | .97 cc. |
| 18 | .02 cc. | .98 cc. |
| 19 | .01 cc. | .99 cc. |
| 20 | 0.00 cc. | 1.00 cc. |

To each tube of this system were added, with the same pipette, two drops of the suspension of red corpuscles. The hemolytic system was placed in the water-bath at 38° C. for four hours, during which it was shaken several times. When taken out of the water-bath, the readings were made after half an hour of sedimentation. In describing our results, we shall refer to the tube numbers given in the above table.

### *Results of the Hæmolytic Tests with Saponin in Normal Rabbits*

By normal, we mean the rabbits having not yet received an injection of saponin and those which did not present at autopsy any lesions of coccidiosis or other parasitic infection.

| No. of rabbit | Date | Hæmol. begins in tube | Hæm. complete in tube |
|---|---|---|---|
| 23 | May 21 | 13 | 12 |
| 24 | May 21 | 13 | 12 |
| 30 | May 21 | 13 | 12 |
| 30 | May 28 | 13 | 12 |
| 30 | May 31 | 13 | 11 |
| 38 | June 30 | 13 | 12 |

These results indicate that the erythrocytes of different rabbits differ only slightly as regards their resistance to saponin. It also shows that resistance remains fairly constant in the same rabbit (Rabbit No. 30).

Results of the hæmolytic tests with saponin in rabbits having received intravenous injections of saponin.

| No. of rabbit | Before Injection | | After Injection | | *No. of injections received* |
|---|---|---|---|---|---|
| | Hæm. begins in tube | Hæm. complete in tube | Hæm. begins in tube | Hæm. complete in tube | |
| 21 | 13 | 12 | 13 | 12 | 6 inj. 4.2 mg. in 7 days |
| 24 | 13 | 12 | 13 | 12 | 3 inj. 5.8 mg. in 4 days |
| 33 | | | 13 | 11 | 11 inj. 1 mg. in 15 days |
| 25 | | | 13 | 12 | 10 inj. 5.8 mg. in 10 days |

### *Hæmolytic Tests with Saponin in Splenectomized Rabbits*

The rabbits of the following table had not received, when the test was made, any injection of saponin.

| No. of Rabbits | Condition | Hæmolysis begins in tube | Hæmolysis complete in tube |
|---|---|---|---|
| 24 | Normal | 13 | 12 |
| 29 | Splenectomized 2 days ago | 14 | 12 |
| 27 | " 7 days ago | 13 | 12 |
| 8 | " 7 days ago | 13 | 12 |
| 19 | " 11 days ago | 13 | 11 |
| 29 | Splenectomized 12 days ago | 13 | 11 |
| 27 | " 15 days ago | 13 | 11 |
| 19 | " 18 days ago | 14 | 12 |
| 19 | Splenectomized 48 days ago | 14 | 11 |
| 38 | Normal | 13 | 12 |

Although Rabbit No. 19 did not show any increased resistance to saponin, it had shown, six days before the last test, an increased resistance of its red cells to hypotonic salt solutions, as can be seen from the following table. This system was prepared so that—

Tube 1 contained 20 drops of physiological salt-solution,
Tube 2 contained 19 drops of physiological salt-solution plus 1 drop of distilled water,
Tube 3 contained 18 drops of physiological salt solution plus 2 drops of distilled water,
Tube 20 contained 1 drop of physiological salt solution plus 19 drops of distilled water.
To each tube of this system two drops of a 3% suspension of the washed red cells were added.

### *Hæmolytic Test with Hypotonic Salt Solution in a Splenectomized Rabbit*

| No. of rabbits | Condition | Hæmolysis begins at tube | Hæmolysis complete in tube |
|---|---|---|---|
| 38 | Normal | 9 | 14 |
| 37 | Normal | 9 | 14 |
| 19 | Splenectomized 42 days ago | 11 | 15 |

In summing up these results we are permitted to draw only the following conclusions:

(1) After intravenous injections of saponin in rabbits, the resistance of red cells to this drug does not show any marked change.

(2) The red cells of splenectomized rabbits are not different in their resistance to saponin from those of normal rabbits, although they are more resistant to hypotonic salt solution.

This is not completely in accord with what Pearce found in his splenectomized dogs in which increased resistance of red corpuscles to saponin as well as to hypotonic solutions and other hemolytic substances occurred. They do not differ, however, from the observations of McNeil in man.

Although it is not our purpose to discuss at length the question of hæmolysis by saponin, this part of our results

convinced us that, as far as the corpuscles alone were concerned, the destructive power of saponin remains fairly constant when used in the same amounts and the same dilutions. We know, however, that the action of saponin *in vivo* is far more complex than that shown in a test-tube with washed corpuscles. Ranson found that cholesterin and lecithins, in salt solution, have a protective power against hemolysis by saponin. Clark and Evans have recently shown that normal human serum shows a protective power against hemolysis by saponin far more active than the protective power of cholesterin and lecithin; they were, however, unable to tell to what mechanism or substance this normally present protective power of serum against hemolysis by saponin was due.

We have not made any study along these lines and have not investigated the chemistry of the blood of our rabbits before injection. We had the impression, however, that in our series of rabbits two pregnant animals showed a greater resistance to saponin. Receiving the same amount of saponin intravenously, they became less rapidly anæmic, and this was due certainly to a diminished destruction of corpuscles, because the hæmopoietic reaction, found at autopsy, was less marked in these animals than in the controls. For this reason, and to maintain more constant conditions, we used later only male rabbits which, being all fed in the same manner, would have probably a more constant amount of lipoids in their blood.

### Action of Saponin on Blood Platelets

As we found, in our first rabbits injected with saponin, the liver and spleen filled with megalocaryocytes, we were naturally forced to consider the action of this drug on platelets. Indeed, most of the hematologists in Europe as in this country, agree at present with Wright's doctrine of the megalocaryocytic origin of blood platelets (Naegeli, Schridde, Cesaris-Demel, Ferrata, Aschoff, Ogata, Downey, Klein, Foa, Brown, Lesourd and Pagniez, Di Guglielmo, and others), although some Italian investigators, for example, Pianese and Perroncito, have rejected it recently.

Consequently we were inclined to think that a careful investigation of the platelet curve of our injected rabbits would be of some use.

*Technique.* In order to avoid, as far as possible, any causes of error, we were always careful to draw the sample of blood from the first drop of blood arriving freely after the puncture of the marginal vein of the ear of the rabbit. In drawing the blood into the red counting pipette, the tip of the pipette was held in the middle of this drop and did not touch the lips of the vascular wound. Then it was rapidly diluted in the pipette with physiological salt solution or more often with the following fluid: 30 cc. of a 3% solution of sodium citrate in distilled water and 1 c.c. of a 1:150 brilliant-cresyl-blue solution in distilled water, the two fluids being mixed and filtered before use.

As soon as the pipette was filled with blood, it was shaken in the electrical shaker. If this is done rapidly, one succeeds almost always in avoiding any agglutination of platelets. The very few samples in which agglutination was observed were discarded as not reliable for accurate counting. After ten minutes' shaking, the drop for examination was allowed to deposit for twenty minutes, which is the time required for a complete sedimentation of the platelets. Let us add that all counts have been made with the same pipette and same chamber, by the same observer, and were often controlled by the other.

The following table shows the effect upon the platelets of the injection of large doses of saponin.

TABLE I.

Reduction of platelet-count after injection of saponin.

| Rabbit | Date Time | Saponin injected | Erythrocytes per cu. mm. | Platelets per cu. mm. |
|---|---|---|---|---|
| 40 | June 29— | | | |
| | 9 a.m. | ——— | 7,776,000 | 752,000 |
| | 9:45 a.m. | 15 mgm. in 15 cc. phys. salt solution | | |
| | 10:15 a.m. | ——— | 6,400,000 | 496,000 |
| | 2 p.m. | ——— | 5,850,000 | 200,000 |
| | 4:15 p.m. | ——— | 5,800,000 | 160,000 |
| | On June 30th the animal was found dead. | | | |
| 41 | July 1— | | | |
| | 9 a.m. | ——— | 6,100,000 | 532,000 |
| | 10:30 a.m. | 15 mgm. in 7.5 cc. phys. salt solution | | |
| | 12:30 p.m. | ——— | 4,800,000 | 320,000 |
| | 2:30 p.m. | ——— | 4,936,000 | 290,000 |
| | 4:30 p.m. | ——— | 5,008,000 | 272,000 |
| | On July 2nd the animal was found dead. | | | |

Similar observations were frequently made during our experiments. They show plainly that there is a diminution of the number of platelets in the circulating blood, following an injection of saponin.

We had to keep in mind, however, that several substances have been used to diminish temporarily the blood platelets of the circulating blood. Quite recently Roskam succeeded in doing so in dogs, with injections of gelatin. While one does not know exactly what becomes of these platelets, one might wonder if they were not stored up in the visceral circulation.

Two rabbits were used to answer partly this question as regards saponin. After injection of saponin they were anesthetized with ether, and laparotomized; simultaneously samples of blood were taken by puncture of the vein of the ear and of the portal or splenic vein.

These experiments, while very concordant, show that the number of platelets circulating in the blood of the portal system is not higher than the number of platelets

TABLE II.

Platelet counts in peripheral and visceral blood.

| Rabbit | Date Time | Saponin injected | Peripheral blood vein of ear | | Visceral blood Portal and Splenic veins | |
|---|---|---|---|---|---|---|
| | | | Erythrocytes per cu.m. | Platelets per cu.m. | Erythrocytes per cu.m. | Platelets per cu.m. |
| 39 | June 28— | | | | | |
| | a.m. | ——— | 6,576,000 | 670,000 | | |
| | 1:15 p.m. | 25 mgm. in 20 cc. phys. salt solution | | | | |
| | | | | | Portal Vein | |
| | 2:45 p.m. | ——— | 6,070,000 | 360,000 | | |
| | 4:45 p.m. | ——— | 4,800,000 | 328,000 | 4,832,000 | 304,000 |
| 44 | July 11— | | | | | |
| | 8:30 a.m. | ——— | 6,626,000 | 782,000 | | |
| | 9:15 a.m. | 15 mgm. in 5 cc. phys. salt solution | | | | |
| | 11:20 a.m. | ——— | 6,400,000 | 342,000 | | |
| | 1:15 p.m. | ——— | 6,144,000 | 280,000 | | |
| | 4 p.m. | ——— | 6,648,000 | 220,000 | Splenic Vein | |
| | 5 p.m. | ——— | | | 4,996,000 | 200,000 |

in the peripheral blood. They do not allow us, however, to discard the hypothesis that blood platelets are retained in the capillaries of an organ or even of the whole body.

A real proof that we are dealing with a destruction of platelets would be to obtain this destruction *in vitro*, and a series of experiments was planned to see if this could be done.

TABLE III.

Action of saponin *in vitro*.

| Rabbit | I. Saponin solution: 0.25 mgm. per 1 cc. 0.9% NaCl | II. 0.9% NaCl solution | Corresponding tube in hæmolytic system | Platelets per cu.mm. | |
|---|---|---|---|---|---|
| | cc. | cc. | | | |
| 42 | | 1 | | 428,000 | |
| | 0.035 | 0.965 | | 262,000 | |
| | 0.04 | 0.96 | 16 | 260,000 | |
| | 0.045 | 0.955 | | 240,000 | |
| | 0.05 | 0.95 | 15 | 104,000 | |
| | 0.055 | 0.945 | | 100,000 | |
| | 0.06 | 0.94 | 14 | 36,000 | |
| | 0.07 | 0.93 | 13 | 48,000 | |
| | 0.075 | 0.925 | | 40,000 | |
| 44 | | II. Sodium citrate and brilliant cresyl blue | | Platelets | Erythrocytes |
| | | 1 cc. | | 420,000 | 7,936,000 |
| | 0.06 cc. | 0.94 cc. | 14 | 148,000 | 7,736,000 |
| | 0.07 cc. | 0.93 cc. | 13 | 172,000 | 6,584,000 |
| Another sample of blood, other pipette and chamber | 0.06 cc. | 1 cc.<br>0.94 cc. | 14 | 488,000<br>196,000 | 6,080,000<br>5,584,000 |
| Another sample of blood | 0.08 cc. | 1 cc.<br>0.92 cc. | 12 | 496,000<br>168,000 | 6,968,000<br>6,112,000 |
| Another sample of blood | 0.09 cc. | 1 cc.<br>0.91 cc. | 11 | 364,000<br>176,000 | 5,824,000<br>5,808,000 |

*Technique:* Several samples of blood, drawn from the same drop, were taken in several pipettes; one with ordinary 0.9% salt solution or with the fluid used for platelets (see above); others with physiological salt solution to which had been added a certain amount of a solution of saponin containing .25 mg. to 1 c.c. of salt solution. These last diluting fluids were measured so that hæmolysis could not be obtained at all or could be obtained only after a long exposure. The strength of these dilutions were known by our previous hæmolytic tests. When all the pipettes were filled with these special diluting fluids, they were shaken in the electrical shaker. As twenty minutes were required for the sedimentation of the drop in the counting chamber before any counts of platelets were made, the platelets were exposed to the action of saponin, *in vitro*, for about 30 minutes.

Of course, none of the rabbits used in these experiments had received previously any injection of saponin.

This demonstrates that saponin in physiological salt solution, in a dilution which hæmolyses red cells slowly or not at all, destroys many of the platelets. It is probable, therefore, that the platelets do not all show *in vitro* the same resistance to hæmolytic agents, because some survive while others are destroyed just as in the case of the red corpuscles, for it is well known that young red cells, more especially nucleated red cells, have a higher power of resistance than mature erythrocytes.

While Bunting deserves entire credit for the discovery of this action of saponin, we think that a report of our experiments may be useful, because they seem to meet, more clearly than Bunting's, the objections which may be raised against the statement that saponin destroys the platelets. Several investigators have attempted to withdraw all the platelets from the circulating blood by means of injections of such substances as gelatin. This is effective in causing their disappearance, but we do not know how it acts nor whether the platelets are really destroyed. The actual destruction of platelets would stimulate their specific regeneration in the hæmopoietic organs, while this would probably not follow the action of any agent which only temporarily withdraws platelets from the peripheral blood.

### *Process of Regeneration*

Our rabbits injected with saponin did not all show the same clinical picture. It is well known that besides a toxic action, not always measurable and leaving no lesions, saponin destroys the corpuscles in the circulating blood, and, also, as Bunting and the writers have shown, destroys the blood platelets. Thus the effect of saponin, like that of any other poison, is subjected to many factors which are probably not all known and not measurable, depending on the individual resistance, the concentration of the dose, its excretion from the body, *et cetera*.

But as far as its action on the blood curve is concerned, we had an impression that in general our rabbits behaved in two different ways; some receiving doses of saponin became rapidly anemic, lost weight and appetite and died after a rather short time with a high degree of anemia (rabbits 3, 5, 19, 38); others, receiving somewhat smaller doses (per body weight) or the same doses but less often repeated, showed in the beginning also anemia, loss of weight and appetite. They then entered a period where the same doses of poison did not seem to have the same effect any more on their blood picture; the red blood cell count became higher again; the rabbits, more lively, seemed to have come to a stage of equilibrium in which their hæmopoietic organs were able to restore daily the blood elements destroyed (rabbits 11, 12, and others). On the contrary, the rabbits of the first category, while their hæmopoietic organs showed a tremendous attempt to repair their losses, were unable to reach before death this stage of equilibrium.

In the rabbits which had received injections of saponin during long periods, there was an adaptation of the activity of the hæmopoietic organs to the losses of blood, and this was made still more clear in the rabbits which did not receive regularly the same dose. These, while running a long course, became irregularly anemic, but never were able to reach the stage of equilibrium above mentioned (rabbits 1 and 4).

The rabbits of another group received a dose strong enough to kill them in less than twenty-four hours, so that their hæmopoietic organs had not had time to show any tendency to repair the loss of blood (rabbits 39, 40, 41). Consequently, we shall present separately the records of these three groups.

*Rabbits 39, 40 and 41:* All these rabbits were injected with large doses of saponin from 15 mgr. to 30 mgr. All of them died in less than twenty-four hours after the injection. The autopsy findings were all alike: liver and spleen normal; hyperemia of the bone marrow with numerous diffuse and small hæmorrhages; no increase of cellular activity in the bone marrow but a large number of megalocaryocytes. While the spleen, the liver and other organs show no pathological changes, the bone marrow even so shortly after the injection (four or five hours for rabbit 39) shows a marked dilatation of its capillaries filled with blood and diffuse hæmorrhages.

We shall describe the protocols of three rabbits of the second group which had shown tremendous restorative efforts of the hæmopoietic organs.

AUTOPSY (performed immediately: Bone marrow dark red. Microscopically: *Spleen.* Tremendous myeloid metaplasia with normoblasts, myelocytes and especially megalocaryocytes (see Fig. No. 1). These are proportionally more abundant than in any other myeloid tissue; this is so well marked that one seems to deal with a genuine megalocaryocytic reaction. These megalocaryocytes

PROTOCOL OF RABBIT No. 3

(Old, long hair)

| Date Time 1921 | Saponin injected intravenously | Erythrocytes | Leucocytes | Hæmoglobin | Observations |
|---|---|---|---|---|---|
| Mar. 10— | | | | | |
| 3 p.m. | ——— | 4,084,000 | 11,400 | 104% | |
| 4 p.m. | 5 mgm. in 2.5 cc. phys. salt sol. | —— | — | — | |
| Mar. 11— | | | | | |
| 3 p.m. | ——— | 2,416,000 | 44,000 | | |
| 4 p.m. | 5 mgm. in 2.5 cc. phys. salt sol. | —— | — | — | |
| Mar. 12— | | | | | |
| 1 p.m. | 4 mgm. in 2 cc. phys. salt sol. | —— | — | — | Paraplegia of hind legs |
| Mar. 14— | | | | | |
| 11 a.m. | ——— | 400,00 | 10,200 | 85% | |
| Mar. 14— | | | | | |
| 12 a.m. | ——— | —— | — | — | The animal dies |

show numerous figures of Wright and marked phagocytosis of polymorphonuclear leucocytes with pseudo-eosinophilic granules and small lymphocytes. All the myelocytes found have pseudo-eosinophilic granulations; no basophilic myelocytes are seen. Many macrophages containing granules of pigment exist throughout the splenic pulp. Many free megalocaryocytes, myelocytes and normoblasts are in the large splenic sinuses and splenic veins.

*Liver:* Many megalocaryocytes in the capillaries (see Figs. 2 and 3). Most of them probably come from the spleen and stop in the hepatic circulation. Some may be formed in the liver itself. Also, in the hepatic circulation there are myelocytes and normoblasts which have surely the same origin as the megalocaryocytes.

*Lung:* Myelocytes and megalocaryocytes in the capillaries and in the pulmonary vessels. The megalocaryocytes are almost devoid of cytoplasm.

*Adrenal:* A few megalocaryocytes in the capillaries (see Fig. 4).

*Kidney:* Normal.

*Bone marrow:* Hyperplasia with many normoblasts and erythrocytes and many megalocaryocytes, which show a great tendency to phagocytosis. Hyperæmia and hæmorrhages.

*Rabbit No. 5.* This rabbit showed exactly the same clinical picture; became rapidly anæmic, lost 300 grams and died after five days.

Two and three days after the beginning of the injection, the number of normoblasts in the circulating blood was very high. The autopsy findings are exactly the same as for Rabbit No. 3. Abundant hæmorrhages in the bone marrow.

These two typical autopsies show how rapidly the rabbits become anæmic during the first days after injection. They died without having reached any equilibrium be-

tween the losses of blood-elements and the repairing process, although there is a great activity in the hæmopoietic organs (bone-marrow, spleen and liver). Megalocaryocytes are very abundant in all these organs. Hæmorrhages of the bone-marrow are more numerous than they were in the first group described.

It is interesting to notice that, even when the anæmia occurs less rapidly, the hæmopoietic reaction to saponin injections seems, in animals killed at the same period, quite as marked. This is shown by the following record:

PROTOCOL OF RABBIT No. 24

(Weight 1940 grams; female, pregnant)

| Date Time | Saponin injected intravenously; 4 mgm. per kilogram of body weight | Erythrocytes | Leucocytes | Hæmoglobin per cent |
|---|---|---|---|---|
| May 17— | | | | |
| —— | —— | 7,168,000 | 13,200 | 101 |
| May 22— | | | | |
| 10 a.m. | 5.8 mgm. in 1.41 cc. phys. salt sol. | —— | —— | —— |
| May 23— | | | | |
| 10 a.m. | Similar injection | —— | —— | —— |
| May 24— | | | | |
| 10 a.m. | Similar injection | —— | —— | —— |
| May 25— | | | | |
| 10 a.m. | —— | 5,568,000 | 15,200 | 71 |

The rabbit is killed at 12 m., being in excellent condition.

AUTOPSY: *Spleen*—numerous megalocaryocytes and several myelocytes.

*Liver:* Megalocaryocytes, showing phagocytosis, and very few myelocytes in the capillaries.

*Lung:* Megalocaryocytes, in the capillaries, almost deprived of their cytoplasm.

*Bone marrow:* Hyperæmia; hyperplasia with many megalocaryocytes.

*Lymph gland:* Normal; no megalocaryocytes or myelocytes.

*Adrenal:* No megalocaryocytes.

While the autopsy findings of the three last records are almost the same, it must be noticed that Rabbit No. 24 became much less anæmic than Rabbits 3 and 5; this may have occurred because it was pregnant and in this condition, on account of the increased cholesterinæmia, the protective power of the blood serum against hæmolysis by saponin may be increased.

Before we describe the case of a rabbit, in which an equilibrium between the destructive and repairing processes is reached, we shall present a case where, although the animal remained living for about twenty days, the anæmia was irregularly progressive and no equilibrium was ever reached. This was due to the irregularity of the amount injected.

PROTOCOL OF RABBIT No. 4

(Weight 2375 grams on March 16th)

| Date Time 1921 | Saponin injected intravenously | Erythrocytes per cu.mm. | Normoblasts per cu.mm. | Leucocytes per cu.mm. | globin per cent |
|---|---|---|---|---|---|
| March 16 | —— | 5,600,000 | — | 12,000 | — |
| 10 a.m. | 6 mgm. in 2 cc. phys. salt sol. | —— | — | — | — |
| March 17 | —— | 3,348,000 | 376 | 37,600 | 85 |
| 10 a.m. | Similar injection | —— | — | — | — |
| March 18 | —— | 2,357,000 | — | 43,000 | 78 |
| 10 a.m. | Similar injection | —— | — | — | — |
| March 19 | —— | 2,048,000 | 2480 | 24,800 | 70 |
| 10 a.m. | Similar injection | —— | — | — | — |
| March 21 | —— | 816,000 | 327 | 10,900 | 60 |
| 10 a.m. | 7.5 mgm. in 2.5 cc. phys. salt sol. | —— | — | — | — |
| March 22 | —— | 720,000 | 792 | 13,200 | 60 |
| 12 a.m. | Similar injection | —— | — | — | — |
| March 23 | —— | 690,000 | 1026 | 18,000 | 52 |
| 11 a.m. | Similar injection | —— | — | — | — |
| March 26 | —— | 708,000 | 790 | 15,000 | 40 |
| 10 a.m. | Similar injection | —— | — | — | — |
| March 29 | —— | 972,000 | 455 | 9,100 | 50 |
| 10 a.m. | 6 mgm. in 2 cc. phys. salt sol. | —— | — | — | — |
| March 30 | —— | 786,000 | 315 | 10,500 | 48 |
| 11 a.m. | 8 mgm. in 2 cc. phys. salt sol. | —— | — | — | — |
| Mar. 31-12 | 10 mgm. in 2.5 cc. phys. salt sol. | —— | 131 | — | — |
| April 1 | —— | 600,000 | 82 | 8,200 | 45 |
| Weight 1790 gms. | | | | | |
| 11 a.m. | Similar injection | —— | — | — | — |
| April 2 | —— | 436,000 | 968 | 8,600 | 45 |
| 10 a.m. | Similar injection | —— | — | — | — |
| April 4 | —— | 760,000 | 396 | 13,200 | 47 |
| 11 a.m. | Similar injection | —— | — | — | — |

On April 5th the animal was found dead.

AUTOPSY: Weight—1320 grams. The inferior part of the bone marrow of the long bones is red and the superior part is pinkish gray. Liver and spleen enlarged. Some lymph glands also show a slight increase in size.

*Microscopically:* Liver—necrotic foci; slight myeloid reaction and exaggeration of the connective tissue.

*Spleen:* A greater myeloid reaction with numerous megalocaryocytes.

*Adrenals:* Some megalocaryocytes situated between the cortical and chromaffine tissue.

*Bone marrow:* Well defined focus of necrosis resembling a fresh infarct. Hyperplasia of moderate degree.

We shall describe now two rabbits presenting the typical history of those where an equilibrium between the effects of blood destruction and blood repair is reached. The second rabbit described was splenectomised and shows a very interesting blood regeneration.

PROTOCOL OF RABBIT No. 12

(Weight on April 29th—2340 grams)

| Date Time 1921 | Saponin injected intravenously 4 mgm. for body weight | Platelets per cu.mm. | Erythrocytes per cu.mm. | Leucocytes per cu.mm. | Hæmoglobin per cent |
|---|---|---|---|---|---|
| April 23 | —— | —— | 6,384,000 | 5,900 | 102 |
| April 29 | —— | —— | 5,632,000 | 9,200 | 93 |
| April 30 | —— | 580,000 | —— | — | — |
| 2 p.m. | 5.6 mgm. in 1.4 cc. phys. salt sol. | —— | —— | — | — |
| May 2 | —— | 112,000 | 2,567,000 | 30,000 | 71 |
| 10 a.m. | Similar injection | —— | —— | — | — |
| May 3, 4 p.m. | Similar injection | —— | —— | — | — |
| May 4 | —— | 250,000 | 1,392,000 | 8,000 | 65 |
| May 5 | Similar injection | —— | —— | — | — |
| May 6 | —— | —— | 2,400,000 | 6,200 | 50 |
| 12 a.m. | Similar injection | —— | —— | — | — |
| May 9 | —— | 396,000 | 3,328,000 | 4,400 | 50 |
| 12 a.m. | Similar injection | —— | —— | — | — |
| May 10 12 a.m. | Similar injection | —— | —— | — | 65 |
| May 11 | —— | 386,000 | 3,936,000 | 10,000 | 65 |
| 12 a.m. | Similar injection | —— | —— | — | — |

On May 11, at 4 p.m., the animal died.

AUTOPSY: The bone marrow is firm, pinkish-gray in some places and grayish-white in others.

*Microscopically:* The *spleen* shows numerous megalocaryocytes and numerous macrophages loaded with pigment.

The *liver* shows pigmentation of the hepatic cells and a few megalocaryocytes.

The *bone-marrow* shows hyperplasia, hyperæmia, hæmorrhages (see Fig. No. 5) and foci of necrosis where there is a beginning of organization.

In the *lungs* there are some megalocaryocytes almost without cytoplasm.

PROTOCOL OF RABBIT No. 11

At 3 p. m. the animal was in very good condition. Then it was killed as a control for Rabbit No. 12 whose spleen had not been removed.

AUTOPSY: *Liver:* A few megalocaryocytes, in the capillaries; almost no myelocytes. Pigment in the hepatic cells.

*Adrenal:* Normal.

*Lymph nodes:* Several lymph nodes show myeloid reaction with numerous megalocaryocytes, several showing phagocytoses. (See Fig. 6.)

*Lung:* A few foci of hæmorrhages.

*Bone marrow:* Hyperplastic almost everywhere, with all kinds of myeloid cells (myelocytes, normoblasts, but more especially, many megalocaryocytes). Several megalocaryocytes show figures of Wright and phagocytosis. Other portions of the bone marrow are not very hyperplastic and remain fatty; even here, however, the most

| Date Time 1921 | Saponin injected | Hæmoglobin per cent | Platelets per cu.mm. | Erythrocytes per cu.mm. | Leucocytes per cu.mm. | Observations |
|---|---|---|---|---|---|---|
| April 22 | —— | 104 | —— | 6,992,000 | 11,000 | —— |
| April 23 | —— | — | —— | —— | —— | Spleen rem'd |
| April 27 | —— | 105 | —— | 8,096,000 | 6,900 | —— |
| April 30 | —— | 103 | 840,000 | 7,752,000 | —— | wgt.1700 gms |
| 2 p.m. | 4 mgm. in 1 cc. phys. salt solution | — | —— | —— | —— | —— |
| May 2 | —— | 95 | 400,000 | 4,256,000 | 12,000 | —— |
| May 3 4 p.m. | Sim. inj. | — | —— | —— | —— | —— |
| May 4 | —— | 90 | 192,000 | 3,200,000 | 24,000 | —— |
| 3 p.m. | Sim. inj. | — | —— | —— | —— | —— |
| May 5 | Sim. inj. | — | —— | —— | —— | —— |
| May 6 | —— | 72 | —— | 3,232,000 | 16,000 | —— |
| 2 p.m. | Sim. inj. | — | —— | —— | —— | —— |
| May 9 | —— | 90 | —— | 4,800,000 | 11,600 | —— |
| 2 p.m. | Sim. inj. | — | —— | —— | —— | —— |
| May 10 | Sim. inj. | — | —— | —— | —— | —— |
| May 11 | —— | 96 | —— | 5,560,000 | 6,000 | —— |
| 2 p.m. | Sim. inj. | — | —— | —— | —— | —— |
| May 12 | —— | — | —— | —— | —— | wgt.1600 gms |
| May 13 | —— | 100 | —— | 6,008,000 | 6,400 | —— |
| 2 p.m. | Sim. inj. | — | —— | —— | —— | —— |
| May 14 | Sim. inj. | — | —— | —— | —— | —— |
| May 15 | Sim. inj. | — | —— | —— | —— | —— |
| May 16 | Sim. inj. | — | 640,000 | —— | —— | —— |
| May 17 | —— | — | —— | 7,392,000 | —— | —— |
| 1 p.m. | Sim. inj. | — | —— | —— | —— | —— |

numerous cells, among the fat cells, are megalocaryocytes. Scattered throughout the organ are numerous foci of hæmorrhages; other portions show young granulation tissue with fibroblasts beginning to elaborate connective fibers.

SUMMARY AND DISCUSSION

We have studied rabbits which had been injected with large doses of saponin, others with small doses, the doses remaining the same or being changed at each injection. We have studied rabbits, so treated, during a few hours, others during a few days, still others during a few weeks (Rabbit No. 33, one month). Some rabbits were splenectomized. *We have never found an aplasia of the bone-marrow.*

On the contrary, the preceding protocols demonstrate that while on the day of the first injection there is no appreciable hæmopoietic activity, a few days later (3, 4 or 6 days) the bone-marrow is hyperplastic and that also in the spleen and the liver a metaplasia of myeloid elements takes place. Let us insist on the fact that in adult rabbits the presence of myeloid elements between the fat cells does not mean necessarily hyperplasia, as it would in man. Normally the adult rabbit has a bone marrow with a fairly large amount of myeloid cells, as Roger and Josué had shown some time ago and as everyone familiar with blood formation in rabbits knows. It is necessary to compare almost the entire bone marrow of the experimental rabbit with that of the normal to reach a decision as to the presence or absence of hyperplasia.

This being known, we observed, however, on the third, fourth and fifth days following the injection, a definite hyperplasia of the marrow, but a disturbing factor to this hyperplasia was always present: hæmorrhages occurred following a marked hyperæmia of all the capillaries. (See Fig. 7). These hæmorrhages in the bone marrow are the only lesion found during the first day after injection of saponin and this leads us to believe that at the start there is an impairment in the circulation and consequently in the function of the bone marrow. This is probably the reason why myeloid metaplasia occurs so soon in other organs (spleen, liver, and in certain conditions in lymph-nodes). In order to repair the losses of blood-cells, a complementary blood-forming tissue is needed, as the normal myeloid tissue is unable to develop with full strength.

Isaac and Möckel raised the question whether such a condition, which they also saw in experimental poisoning by saponin, could not be compared to myeloid aleukæmic leukæmia. In fact, when we saw the tremendous metaplasia described in Rabbit No. 3, for example, without much evidence of myeloid elements in the circulating blood, the same comparison came into our minds. This seems, however, less reasonable than the explanation of a vicarious blood formation due to functionally impaired bone marrow. Indeed, in using saponin we produce above all an erythropathia. As saponin has practically no action on leucocytes, it seems improbable that the reaction to such a poison would resemble one of the diseases involving especially white cells.

The myeloid metaplasia, taking place outside of the bone marrow, is strikingly a reaction to the loss of blood platelets and red cells which are the elements destroyed. Megalocaryocytes and normoblasts are proportionally the most numerous elements of that myeloplastic tissue. Of course, there are some myelocytes, but their number is conspicuously low.

*Megalocaryocytic reaction.* Such a statement cannot be made without considering somewhat carefully the question of megalocaryocytic reaction. Dominici, Pianese and others, studying many sections of the spleen and liver of several normal animals, found in all of them a few megalocaryocytes. Some of the sections of these organs, in our normal rabbits, show also occasionally a megalocaryocyte. We speak of megalocaryocytic reaction only when this number is markedly increased (see figures). That this reaction is markedly increased in relation to the destruction of platelets is probable, although we do not find ourselves able to say that no other platelet-repairing mechanism than this exists. Brown and Ferrata have shown that monocytes or cells resembling them could form platelets (monocytoid cells of Ferrata). Bunting is of the opinion that even lymphocytes give rise to blood platelets.

Although we have not in mind to study especially the morphology of megalocaryocytes, we have observed during our experiments the following types of these cells:

*Types of megalocaryocytes:* The megalocaryocytes found in the bone marrow, spleen, liver and other organs of the rabbits injected with saponin, vary in appearance with respect to their size, shape, structure of the cytoplasm and of the nucleus (see Figs. 8 and 9). However, megalocaryocytes described as belonging to different types of cells, may well have been really the result of sections of the same kind of cell made at different levels. This fact must be kept in mind, especially when we are dealing with megalocaryocytes of rabbits, which are far larger than the human megalocaryocytes and in which the nuclei are more complicated. Carnegie Dickson, who made a careful cytological study of the bone marrow, shows a set of drawings made from serial sections of the same megalocaryocyte, where one can see the diversity of aspect of the cytoplasm and nucleus, and may receive the impression that there are various types of cells. Nevertheless, in the saponin-injected rabbits we have seen megalocaryocytes strikingly different in structure. These we may describe before separating them into groups.

These cells vary in size. The smallest are slightly larger than circulating monocytes, while the largest measure about 200$\mu$ in diameter. Generally, the cells are round or oval, but they may be reniform, crescent-shaped, fusiform or almost triangular in outline. Some cells show pseudopod-like processes of varying size, form and number. These amœboid changes can be very well observed in sections of the liver where the megalocaryocytes inside the capillaries are greatly elongated and cylindrical in order to permit their passing through. The outline of the megalocaryocytes is either sharp, coarse or diffuse; sometimes there is a bay-like depression.

Many authors have described the structure of the cytoplasm of the megalocaryocytes and, since the classical work of Wright on blood-platelet formation, this question has been thoroughly and carefully studied. Our findings are closely similar to what has been described. Indeed, there are cells in which the cytoplasm is apparently homogeneous and basophilic. Frequently the cytoplasm of these cells shows one or more homogeneous eosinophilic areas of different size and shape, which may have been produced by the fusion of several phagocyted erythrocytes. Some cells have a more abundant cytoplasm, apparently homogeneous, but less basophilic, often with the same oxyphilic areas above described; while in others the protoplasm is slightly acidophilic. Many cells, however, with a basophilic or slightly eosinophilic cytoplasm, show a granulated structure with the differentiation of zones as described by Wright, Foa, Schridde, Cesaris, Demel, Di Guglielmo, Ferrata and many others. These cells show a cytoplasm generally formed by two zones, usually sharply distinct: the large, granulated,

perinuclear zone and the narrow, apparently homogeneous, peripheral zone, showing a delicate basophilic or a light acidophilic reaction. The granules, very small, round or elongated, are stained lilac or violet, and are generally arranged in more or less definite parallel rows, circumscribing the nucleus. Some cells show an irregular granulation, with small and large granules, the latter generally lying near the periphery of the cell. As these large granules are separated one from another and embedded in a basophilic protoplasm, each granule is surrounded by a delicate zone, bluish in color. The cytoplasm of the megalocaryocytes frequently shows its phagocytic activity by containing several embedded cells; sometimes there are cells lying in small depressions of the cytoplasm, showing the act of engulfing.

The nuclei also present a great variety of shape and size, often due to projection of their sectioned portion, as mentioned above. According to their strikingly different structure, it is easy to distinguish several types:

1. Large nuclei, very complicated in form, unevenly globular and hollow, with some irregular openings that permit the communication of the cytoplasm, situated inside and outside. These nuclei have been compared to a "basket-work," composed of threads twisted in the most complex way. They vary greatly in aspect, according to the level through which the section passes. The sections near the periphery show the nucleus as formed by more or less numerous small pieces grouped together, each one seeming to be limited by a definite membrane. The sections near the centre, through the equator, for instance, show a ring or wreath-shaped nucleus, formed by many nuclear pieces, irregularly joined by their extremities.

The chromatin is dispersed in more or less numerous granules of various size, probably supported by a reticulum of *linin*. These nuclei are full of nuclear juice, or *paralinin*, so that they appear as though formed by deeply stained basophilic granules embedded in a colorless or slightly basophilic substance.

2. Small round nucleus of the same fine structure.

3. Large or small nuclei of various and very irregular forms, such as to defy any description. These nuclei are so rich in chromatin that the entire nuclear body is homogeneously very deeply stained, almost black, giving the idea of irregularly spread ink spots. These are very pyknotic nuclei.

4. A nucleus that appears composed of many short segments of a very dense, deeply stained chromatin, giving the impression of numerous *chromosomes*. Sometimes the thread of dense chromatin seems to be continuous, like a *loose spirem—luto spireme*. The nucleoli in these types of nuclei are sometimes very conspicuous and acidophilic.

These different conditions of the cytoplasm and nucleus of the megalocaryocytes perhaps are the result of different evolutional phases of the same kind of cell.

In resuming, we have seen the following types of cells or probably the various successive evolutional phases of the same type of megalocaryocyte:

1. Small cells with a deeply stained basophilic cytoplasm, apparently homogeneous, and a small round, oval or reniform nucleus, rich in nuclear juice (megalocaryoblast of Ferrata ?).

2. Large cells with an apparently homogeneous and either basophilic (lymphoid megalocaryocyte of Ferrata) or acidophilic cytoplasm, and a complex "basket-work" nucleus, rich in paralinin.

3. Large cells with a granulated cytoplasm and a complex "basket-work" nucleus, rich in nuclear juice (granulated megalocaryocyte of Ferrata).

4. Cells of various sizes with an apparently homogeneous protoplasm and an irregular pyknotic nucleus (degenerate megalocaryocyte of Carnegie Dickson ?).

5. Cells of various sizes, the nucleus of which has the appearance of a mitotic figure—luto spireme—or chromosomes in formation (megalocaryocyte with nucleus in act of dividing or of rearrangement?).

6. Free nuclei, apparently without protoplasm at all. These nuclei are frequently pyknotic but sometimes vesicular.

Our cytological studies have been made with paraffin sections cut at 4.5$\mu$ and stained with Wright's stain.

That the megalocaryocytic reaction is a specific reaction to the most urgent needs of the blood, was shown by our Rabbit No. 11. This rabbit, which had been splenectomized, after having received many injections of saponin had reached a state of equilibrium between the destructive and repairing processes of the blood. When killed, the bone-marrow of this animal presented the usual lesions which impair partially its functional capacity; there was a slight myeloid metaplasia in the liver; but—and this is the interesting point—there was a megalocaryocytic reaction in almost all the lymph nodes studied.

We had never found megalocaryocytes in the lymph nodes of our other rabbits (Rabbits 21, 22, 23, 24, 25). What happened in this case would be explained by those who admit the unicist theory of the secondary blood formation, in the following manner: In the lymph nodes there are cells which cannot be considered morphologically different from the hæmocytoblast (Ferrata) or lymphoidocyte (Pappenheim) of the bone marrow, as has been admitted by Dominici, Maximow, Pappenheim, Ferrata, and others. Usually in the lymph nodes these cells give rise to lymphoid elements and are, as Ferrata clearly expresses it, hæmocytoblasts in lymphoid function; but, like the medullary hæmocytoblasts which produce megalocaryocytes and other myeloid cells in the bone marrow, in special conditions the hæmocytoblasts of the lymph-nodes can produce these megalocaryocytes or other myeloid elements. The myeloid potentiality of the lymph-

nodes has been stimulated, in the case of Rabbit 11 by the absence of the spleen. That the myeloid potentiality of the lymph nodes has been specifically stimulated to form almost only megalocaryocytes, seems to show that the need of these cells or of the elements deriving from them, was the greatest.

### Mechanism of the Disturbance in the Bone Marrow

During the first hours following an injection of saponin, we noticed an extreme dilatation of all the capillaries of the bone marrow, which are packed with blood. This occurs, however, when other blood poisons are injected into rabbits, as, for example, benzol. We confirm this observation on the effect of benzol injected intravenously, on two rabbits (No. 14 and No. 16). After the intravenous injection of saponin, however, this hyperaemia is almost immediately followed by hæmorrhages due to rupture of the overfilled capillary. This does not occur, so far as we know, in benzol poisoning. The question arises whether the rupture of the capillaries may not be due to an injury and weakening of the capillary wall.

It is, however, not easy to understand why this action of saponin is localized only in the capillaries of the bone marrow, since we do not find any hæmorrhages in other organs. Whatever the cause of these hæmorrhages, it may be that all the other changes are consequences or a response to them. Necroses of some portion of the bone marrow are easily understood as a consequence of an impairment of its normal blood-circulation.

Organization of the necrotic foci or the foci of hæmorrhages is also a natural reaction. The general result of this process will be a partial incapacity of the bone marrow, which, in the areas in which the normal circulation is maintained, shows a high degree of blood formation. This is certainly different from a complete aplasia of bone marrow, such as saponin is classically said to produce.

Returning to the history of the case which determined our experimental study of anæmia following saponin poisoning, we are forced to admit that although saponin gives a clinical picture and autopsy findings in rabbits comparable to those given by the above described patient, there are very important differences which have to be emphasized. Like the spleen and liver of the patient, these organs in our rabbits showed an intense megalocaryocytic reaction; like his blood, their blood contained few red cells, numerous normoblasts, very few myelocytes in rare cases, and very few platelets. There are, however, striking differences: whereas the patient had a low color index and a fatty bone marrow, the experimental animals always had a high color index. There was a high grade of hæmoglobinæmia and hæmoglobinuria (as, for instance, Rabbit No. 4). All our rabbits at autopsy had a hyperplastic bone-marrow, although with its normal capacity very much diminished by diffuse hæmorrhages. The pathological problem of the case described concerns the one-sided blood-formation, but cannot be explained by our findings with saponin poisoning.

We have to admit that we were dealing in this case with another etiological factor and another mechanism. If any suggestion might be made, we would say this: it is improbable that any toxic factor in the body would prevent the formation of myeloid tissue where it normally exists, while at the same time it would allow the building up of myeloid tissue in organs where it is not found in the normal condition. Whatever the etiological factor in such a case may be, it is probable that it acts indirectly on the myeloid tissue of the bone marrow. Perhaps a careful study of the vessels and the nerves of the bone marrow, the functions of which are not well known and have not been thoroughly studied, would be of a greater help.

### Conclusions

1. Single or repeated intravenous injections of saponin in rabbits do not change the resistance of the red blood cells to this toxic substance.

2. The erythrocytes of splenectomized rabbits are more resistant to hypotonic salt solution but not to saponin.

3. In addition to its hæmolytic action saponin is a highly destructive agent for blood platelets, both *in vivo* and *in vitro*.

4. The intravenous injection of saponin into rabbits produces in the bone marrow hyperplasia and, simultaneously, numerous diffuse or circumscribed foci of hæmorrhage; there is not a true aplasia.

5. As the function of the bone marrow is impaired by hæmorrhages and their effects, the spleen, liver and other organs show a vicarious myeloid formation. This newly grown myeloid tissue shows an unusually large number of nucleated red blood cells but principally megalocaryocytes—megalocaryocytic reaction.

6. A megalocaryocytic reaction was observed in the lymph glands, after intravenous injection of saponin, in splenectomized but not in non-splenectomized rabbits. This may be explained as a vicarious function.

7. The megalocaryocytes found in the bone marrow, spleen, liver and other organs of the rabbits injected with saponin are not all identical in appearance. While presenting differences in morphology and structure of the nuclei and cytoplasm, they may be different stages of the same type of cell.

8. For a good understanding of the pathological changes in the bone marrow, it is very important to study large longitudinal sections (of almost the whole organ in rabbits) and cross-sections of the marrow of several bones. This is absolutely necessary in the study of the bone marrow of saponin-injected rabbits.

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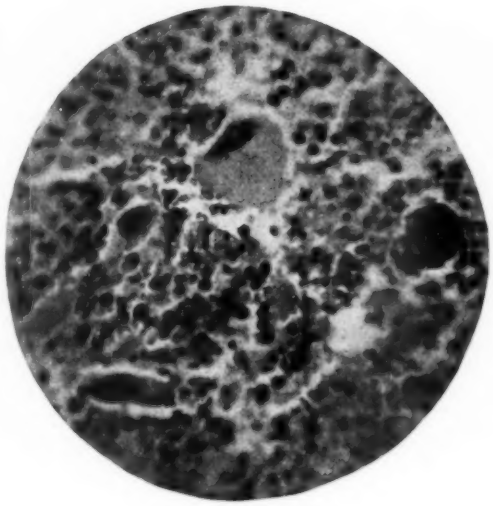

FIG. No. 1

Spleen of rabbit No. 3.

Myeloid reaction with numerous megalokaryocytes.

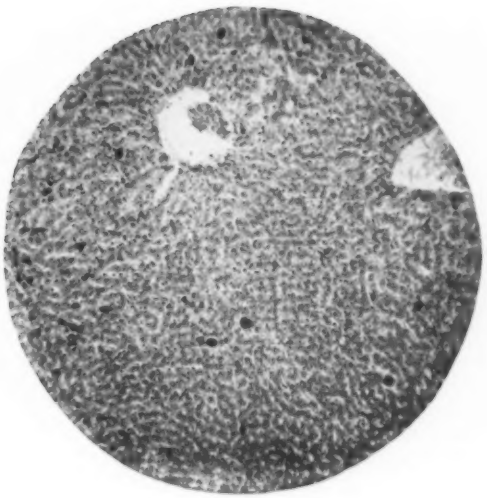

FIG. No. 2

Liver of rabbit No. 3.

Myeloid reaction with numerous megalokaryocytes.

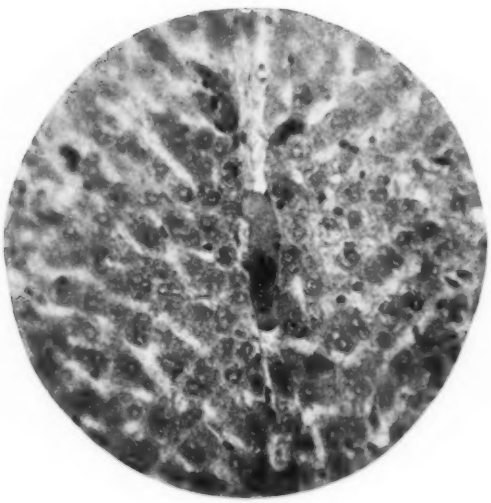

FIG. No. 3

Liver of rabbit No. 3

showing a megalokaryocyte in a capillary

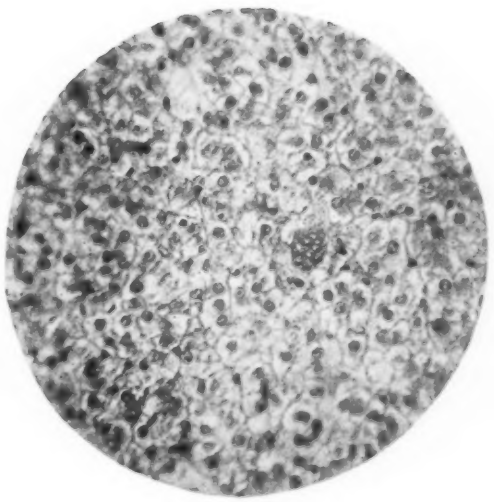

FIG. No. 4

Adrenal of rabbit No. 3

showing a megalokaryocyte in the cortical tissue.

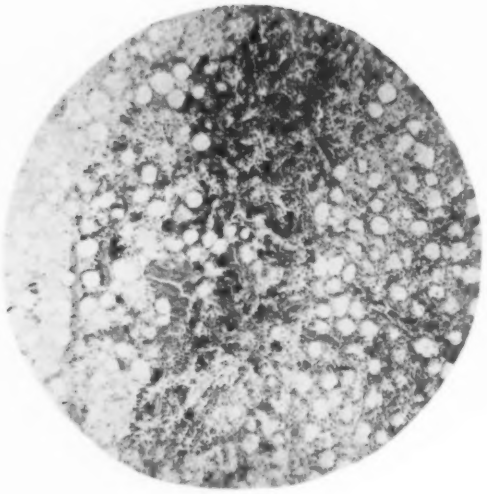

FIG. No. 5

Bone marrow of rabbit No. 12.

Diffuse hæmorrhage and vicarious hyperplasia.

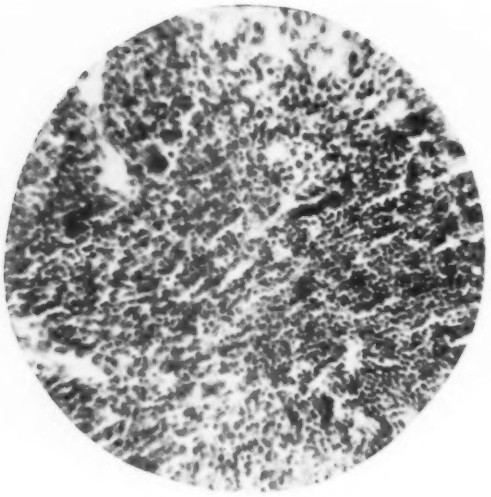

FIG. No. 6
Lymph gland of rabbit No. 11.

Myeloid reaction; a megalokaryocyte is seen in the lymph sinus.

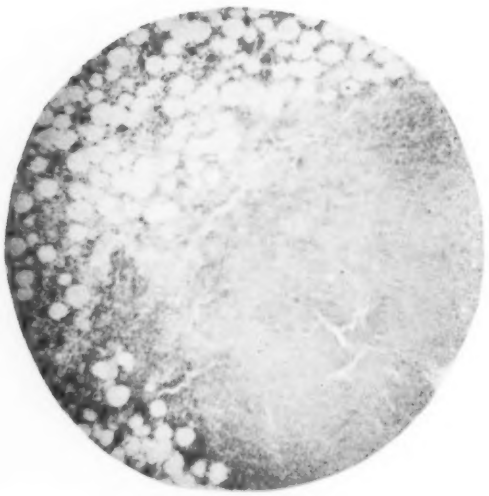

FIG. No. 7

Bone marrow of rabbit No. 22

showing hyperplasia and a large zone of hæmorrhage.

FIG. No. 8
Megalokaryocytes from the spleen of rabbit No. 3 (above) and of rabbit No. 11 (below).

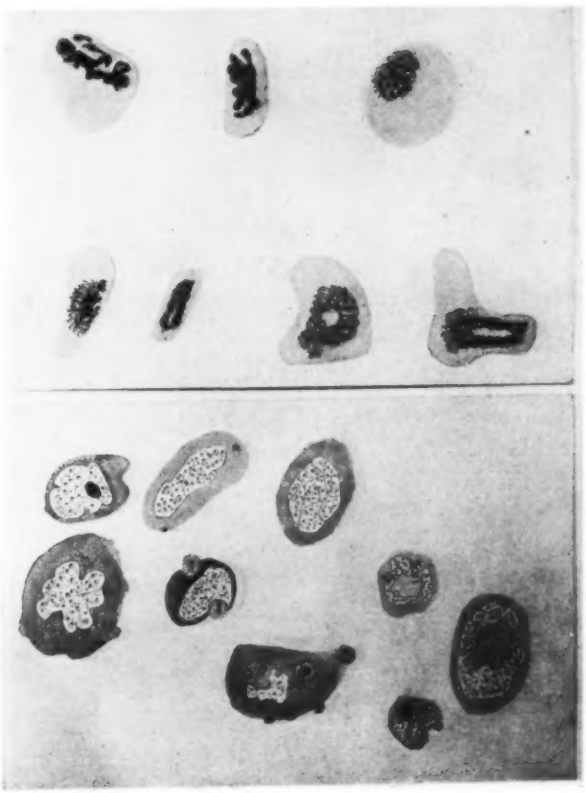

FIG. No. 9
Megalokaryocytes from the spleen (above) and liver (below) of rabbits No. 5 and No. 3.

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# THE SURVIVAL OF CELLS AFTER THE DEATH OF THE ORGANISM

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## Introduction

It is now a matter of common knowledge that cells of certain types can survive for a limited period after removal from the body. The ectoderm, especially noteworthy in this regard, appears to be able to live several days if kept under proper conditions. The test of viability is the "take" of the skin graft. Skeletal, heart, and smooth-muscle fibers can be made to contract and relax for a considerable period of time outside the body. The muscle-fibers in the physiological preparations used for such experiments are supposed to remain alive; the criterion here is the ability of the muscle to contract and relax in a proper artificial environment with suitable stimuli. In muscle-nerve specimens the isolated nerve-fibers are able to conduct stimuli to the muscles for varying periods. Do the axones continue to live, after their separation from the cell bodies, as long as the nerves are able to conduct stimuli to the muscles?

Carrel ('07) succeeded in getting good results from the hetero-transplantation of blood-vessels that had been kept in cold storage for 4 days, while those kept 7, 17, and 20 days, respectively, were not so successfully transplanted. It is not clear from his data whether the cells in the transplanted 4-day blood-vessel remained alive or whether a process of substitution made the transplant a success. In 1910 he succeeded in replanting spleen 44 minutes, and kidney 50 minutes, after removal from the body. The criterion for life in such experiments is the success of the transplantation and the resumption of function of the organ.

Tissue-cultures have shown that most cells not only survive but move about and even multiply for many days after removal from the body. Fischer ('21) cultivated epithelial cells for over two months, while the connective-tissue cells may live and multiply for years (Ebeling, '21). It is probable, therefore, that many types of cells are potentially immortal, given the proper environment. Tissue-culture affords the most advantageous environment that has yet been devised.

In the present study we have endeavored to determine the periods of survival of several different types of cells after the death of the animal, under various conditions other than those of tissue-culture. Our criterion for life was the presence within the cells of certain granules and vacuoles that have a great affinity for neutral red. The value of neutral red as a testing agent was suggested by the following considerations. Experience with tissue-culture has shown that all types of living cells either have or can develop granules and vacuoles with a marked affinity for neutral red. This dye does not become concentrated within the living cells unless there is some pre-existing body or substance to which it can become attached. In the ordinary strengths used it does not stain the cytoplasm or nucleus, nor does it seem to injure the cells. It is a well-known fact that in dead cells of tissue-cultures the granules and vacuoles do not take up neutral red as they do in living cells, although the entire dead cell may stain diffusely if the dye is strong enough. Dead cells, but not living ones, are stained diffusely with various other dyes, such as toluidin blue (Gross, '11; Steckelmacher, '14), and benzidine dyes (Evans and Schulemann, '14), Nile blue B extra and brilliant cresyl blue (Lewis and Lewis, '15).

Granules and vacuoles that have been colored with neutral red in the living cell lose their color when the cell dies (W. H. Lewis, '21). We have noted repeatedly that when the cells of a spread are killed rapidly by heating, the red vacuoles lose their color and the cells become more or less diffusely pink. Much the same results were obtained when the spreads were killed with acetic acid.

As a rule, the dead cells in the various spreads, except those from the intestine, retained their form both as to cytoplasm and nucleus. It was possible to distinguish the dead from the living cells even without the use of neutral red. The main differences are in the texture of the cytoplasm and nucleus and in the character of the nuclear membrane, which becomes more marked after death.

Most cells, under abnormal conditions, develop within their cytoplasm these granules and vacuoles that have an affinity for neutral red. The process takes place not only under the usual conditions of tissue-culture, but even more rapidly under special conditions, such as the introduction of typhoid bacilli into the medium (M. R. Lewis, '20) or the exclusion of dextrose (M. R. Lewis, '21). Similar granules and vacuoles accumulate rapidly in connective-tissue cells and blood-cells, which normally possess few if any, when their supply of oxygen is cut off by sealing the spread under a coverglass (Prigosen, '21). They are also produced in the cells of living animals under the action of numerous acid-azo dyestuffs (Evans

and Scott, '21). We have found that these neutral-red bodies also accumulate in various types of cells that remain alive in the dead animal, or in organs or parts of organs that have been removed, even when they do not appear to be present in the normal, healthy cell. At the death of the animal all the cells of the body are subject to great functional disturbances of one kind or another. With the cessation of heart action all the tissues are immediately deprived of their chief source of oxygen, their food supply, and the usual pathway for the elimination of waste products. Lack of oxygen and food and the accumulation of waste products are probably all concerned in the production of the granules and vacuoles.

In these experiments most of the observations were made on the adult white rat, with a few additional ones on the guinea-pig, rabbit, and man. There were four series of experiments, with numerous repetitions of the individual observations. The rats were killed with ether, chloroform, or a knock on the head. The method of killing caused no variation in the subsequent formation of granules and vacuoles in the cells.

SERIES I. Fourteen rats were used in this series. Various organs were cut out aseptically immediately after death, put into a moist chamber (sterile petri dishes) and kept at 37° F. in an automatically regulated refrigerator for varying periods of time (24, 36, 48, 60, 72, 96, 120, 144, 168, 192, and 240 hours). Small pieces of the organs were then cut up at room temperature in about 5 c.c. of Locke's solution (NaCl .9, CaCl .024, KCl 0.42) without the sodium bicarbonate, plus 1 or 2 drops of a 0.5 per cent solution of neutral red in distilled water. After allowing a few minutes for the dye to penetrate, spreads were examined during the next few hours.

SERIES II (22 rats). Small pieces of tissue were taken from the various organs immediately after death, cut up into minute fragments in the neutral-red solution mentioned above and kept at room temperature (about 70° F.) in the small petri dishes in which they were cut up. In some of the experiments the tissues were handled aseptically. Spreads were examined every few minutes during the first hour or two, then at about one-hour intervals during the remainder of the first 24 hours. The intervals were then lengthened and spreads were examined at about 30, 36, 48, 60, 72, 96, 120, and 144 hours after death.

SERIES III (14 rats). The entire animal was kept at 37° F. for varying periods—6, 12, 24, 36, 48, 72, 96, and 120 hours. Small pieces were then cut up in the neutral-red solution and examined as spreads.

SERIES IV (8 rats). The entire animal was kept at a temperature of about 70° F. for varying periods (1, 2, 6, 9, 12, 18, 24, and 48 hours) after death. Small pieces were then removed and cut up for spreads in the neutral-red solution in the usual manner.

After allowing a few minutes for the dye to diffuse into the minute pieces in each of the above series, the fragments were more or less flattened out under a coverslip in a drop of the fluid, thus forming the spreads for examination. Such spreads can be examined with the oil-immersion; when the granules are first beginning to form, high powers are necessary to recognize them, since they are exceedingly minute. Later, as the granules and vacuoles become larger, they can be recognized with the dry lenses or even with the high power Greenough binocular. The latter type of microscope is very useful for examination of entire organs and large pieces in the dishes. The mechanical injury with resulting death of the cells, produced by cutting the organs with scissors, extends to varying depths in the different organs and tissues composing them, so that along the cut edge there is often a diffuse pink stain. The neutral red does not penetrate very rapidly into the larger pieces; thus the surface may be brilliantly stained while the cells just beneath show scarcely any trace of color. The epithelial lining of the bladder, trachea, and uterus, and the surface of the liver and kidney are very beautiful when the cells become loaded with red vacuoles.

### OBSERVATIONS ON RATS

In the following table are given the maximum intervals after the death of the animal in which red granules and vacuoles were observed in the various types of cells. These figures approach closely the extreme limit of survival under these several conditions. It will be seen that cells survived longest when the organs were cut out and kept at 37° F. (Series I). The cells of tissues cut up into minute fragments and kept at room temperature in Locke's solution without sodium bicarbonate (Series II) survived about as long as when the entire animal was kept at 37° F. (Series III). When the entire animal was kept at room temperature the cells did not survive nearly as long as those in the other three series. It must be borne in mind that there were, as a rule, but few surviving cells in any of the spreads at the extreme time limit and often spreads were observed in which none of the cells contained red vacuoles. It is more than likely that renewed observation and more diligent search would reveal a slightly longer life for most of the cell types mentioned in the table and might considerably increase that for some of the others, as for example, the endothelium in series III.

The table also shows under series II the earliest period at which red granules and vacuoles were observed in the various types of cells. The observations are not complete, but they serve to show that most types of cells begin very quickly after death of the animal to develop granules and vacuoles that have an affinity for neutral red. In smooth muscle, endothelium, and red blood-corpuscles there was sometimes a considerable delay before

any red granules or vacuoles appeared. In the macrophages they were seen at once and appeared very rapidly in most of the other types of cells.

In series I, III and IV, when the organs or animals were kept for some time before examination, the various types of cells probably developed the granules and vacuoles before the tissues were cut up in the neutral-red solution, with the exception of the smooth muscle and endothelium of the animals kept only one or two hours in series IV. Otherwise, in these series it was found that the cells when first examined contained about the maximum number of granules and vacuoles. In series II, however, where the tissues were examined as rapidly as possible after death, there was a gradual increase in the number of red granules and vacuoles in most types of cells during the first few hours until a maximum was attained. This remained more or less stationary for varying periods, according to the type of cell, and then decreased until all color was lost from the granules and vacuoles. Near the end some spreads showed a few surviving cells among thousands of dead ones, while others of the same group contained no living cells at all. No spreads, even those earliest observed, were made up entirely of living cells. Many cells were undoubtedly killed by mechanical injury; most spreads of the various tissues showed areas in which all or most of the cells were dead and other areas in which many cells were alive, that is, contained red granules and vacuoles. There is a comparatively short period near the end when it is difficult to decide whether a cell is alive or dead, but this does not materially influence our results, as we have utilized only the more positive data from the many observations.

TABLE

| SERIES | I. | II. | III. | IV. |
|---|---|---|---|---|
| | Hrs. | Hrs. | Hrs. | Hrs. |
| Large macrophages | 240 | 5′-144 | 120 | 24 |
| Tracheal cartilage | 240 | 20′-144 | 120 | 24 |
| Kidney epithelium | 240 | 10′-120 | 120 | 6 |
| Smooth muscle | 240 | 90′-120 | 12 | 3 |
| Salivary gland epithelium | 192 | 16′-120 | 96 | 3 |
| Bladder epithelium | 192 | 12′-120 | 72 | 3 |
| Tracheal epithelium | 192 | 10′-120 | 120 | 6 |
| Tongue epithelium | 192 | 45′- 48 | 96 | |
| Endothelium | 168 | 35′- 72 | 12 | 3 |
| Small lymphocytes | 168 | 25′- 48 | 60 | 6 |
| Large lymphocytes | 168 | 25′- 48 | 60 | 6 |
| Microcytes | 168 | 25′- 48 | | 3 |
| Lung epithelium | 144 | 35′- 52 | 48 | 3 |
| Leucocytes | 120 | 45′- 24 | 36 | 1 |
| Kupffer cells | 120 | 20′- 49 | 60 | 12 |
| Brain macrophages | 96 | 10′- 48 | 96 | 12 |
| Pancreatic epithelium | 96 | 30′- 49 | 96 | 3 |
| Red Blood-corpuscles | 72 | 53′- 24 | 24 | 1 |
| Liver cells | 96 | 20′- 15 | 12 | |
| Sertoli cells | 96 | 7′- 12 | | 3 |
| Mesenchyme | 72 | 17′- 72 | 48 | 3 |
| Ovarian follicular cells | 72 | 30 | 48 | 1 |
| Uterine epithelium | 72 | 20′- 53 | 12 | 1 |
| Uterine gland cells | 72 | 24 | 12 | 1 |
| Epididymis epithelium | 48 | 55′- 18 | | 3 |
| Adrenal cells | 24 | 15′- 32 | | 3 |
| Fat | 0 | 18 | | 1 |
| Intestinal epithelium | 0 | 60′- 18 | | |
| Nerve cells | 0 | 10′- 1 | | 0 |
| Skeletal muscle | 0 | 0 0 | | 0 |
| Heart muscle | 0 | 0 0 | | 0 |

*Large macrophages*, crowded with red vacuoles, were observed from 24 to 240 hours in series I; in series II, from 5 minutes to 144 hours; in series III, from 6 to 120 hours, and in series IV, from 1 to 24 hours after death. The vacuoles, apparently normal inclusions of these cells, take up the neutral red as rapidly as it penetrates to them. They seem to have the maximum number of vacuoles from the very outset of the observations. The vacuoles do not seem to be of quite the same character as those which develop in most of the other types of cells after the death of the animal, and it is very probable that they do not indicate such a rapid degeneration or injury as do the vacuoles in other types of cells. In series I, the red vacuoles were still present at 240 hours in the macrophages of the diaphragm, tongue, lungs, bladder, trachea, and fat; in the salivary glands at 192 hours; in the heart, thymus, and uterus at 168 hours; in the spleen at 120 hours; and in the epididymis and pancreas at 96 hours. They usually survive as long or longer than most of the other types of cells in the organ. Near the end of their period of survival the number of macrophages with colored vacuoles often decreases, the color changes to a reddish brown, and the distinction between living and dead cells becomes uncertain. The large macrophages were very abundant in the lungs, loose mesenchyme, skeletal muscle, organ capsules, and fat (Figs. 1, 2, and 3). They were fairly numerous in the uterus, bladder, spleen, lymph nodes, thymus, and blood clots, and less so, as a rule, in the heart, ovary, and epididymis. In the kidney, pancreas, salivary glands, testis, and liver they are comparatively rare and probably occur only in the connective septa, so that they were not often seen in spreads. They were not seen in the cortex, cerebellum, cartilage, ectoderm, or adrenal. The macrophages of the cortex and cerebellum and the Kupffer cells of the liver are of a somewhat different character.

*Tracheal cartilage* is one of the most persistent of tissues. In series I red vacuoles were seen in its cells from 24 to 240 hours, in series II from 20 minutes to 144 hours, in series III from 6 to 120 hours, and in series IV at 1 to 24 hours after death. There was a considerable increase in the size of the vacuoles as the period after death lengthened. Macrophages were very abundant in

the peritracheal mesenchyme and in these the red granules and vacuoles usually persisted longer than those in the other cells of the trachea, with the exception of the cartilage.

The *epithelial cells of the kidney tubules* developed many granules and vacuoles in each series (Fig. 6). In series II, in which the development of these granules and vacuoles was followed, there was at first little or no red color, but during the next 3 or 4 hours red granules and vacuoles appeared, gradually increasing in number and size until the cells were crowded with them. This condition remained more or less stationary for varying periods. Red granules and vacuoles were observed in the cells from 24 to 240 hours in series I, from 10 minutes to 120 hours in series II, from 6 to 120 hours in series III, and from 1 to 6 hours in series IV. Most of the spreads showed dead cells diffusely colored, as well as cells containing red vacuoles. The number of cells containing red vacuoles gradually decreased as the period after death lengthened, until only a few could be seen, and finally the color disappeared from these also. The neutral red did not penetrate the larger pieces very rapidly, so that for a time only the uninjured cells on the surface of the larger pieces contained the red vacuoles. In many specimens every cell in the tubules on the normal, uncut, uninjured surface contained vacuoles, while only a part of those on the cut surface showed them, the injured cells being diffuse pink in color. At first only those cells on the superficial side of the tubules on the uncut surface contained red vacuoles, while the deeper ones, to which the neutral red had not penetrated, contained very little color. When, after two or three hours, fresh cuts were made across the larger pieces, the cells in the newly exposed tubules usually showed little or no red color. Vacuoles were undoubtedly present, for the uninjured cells along this surface accumulated neutral red quite rapidly, much more rapidly than when the tissue was first removed.

In the glomeruli red granules were seen, but whether they were in the epithelium, in the endothelium, or in both types of cells could not be determined with certainty. Macrophages are very rare in the kidney.

*Smooth muscle* was noted in the bladder and uterus and occasionally in blood-vessels. This tissue, like endothelium, does not appear to develop vacuoles as rapidly as most of the other tissues. The vacuoles have a characteristic linear arrangement (Fig. 7). In series I, red vacuoles were seen from 24 to 240 hours, in series II from 1½ to 72 hours, and in series III from 6 to 12 hours. Further observations would probably show a longer persistence of vacuoles in series III. In series IV the smooth muscle was examined at 1 and at 3 hours, but no vacuoles could be seen; later, however, spreads from the same dishes did show numerous red vacuoles in the smooth muscle. Either the neutral red had not penetrated or the vacuoles had not developed at the time the first observations were made.

*Salivary-gland epithelium* became heavily loaded with red granules and vacuoles (Fig. 18). There was usually a clear peripheral zone that varied in width in different glands. It was not so pronounced as in the pancreas. The colored vacuoles were crowded in among the secretion granules, which did not take up the neutral red. The number of colored vacuoles seemed to vary in direct proportion to the number of secretion granules. Red granules and vacuoles were found from 24 to 192 hours in series I, from 16 minutes to 120 hours in series II, from 6 to 96 hours in series III, and from 1 to 3 hours in series IV. In series II the duct epithelium was occasionally observed (120 hours) to be loaded with red granules and vacuoles, while very few of the gland cells contained them, most of the latter being dead. Macrophages were not common.

The *bladder epithelium* appears to consist of two rather distinct types of cells. When first examined from 10 to 60 minutes after death in series II, the cells contained only a few small granules and vacuoles, but during the course of the next 3 or 4 hours these increased greatly in both number and size. At this time a marked difference in their reaction to neutral red becomes apparent in the two types of cells. The larger type, often binucleated or multinucleated, developed many granules and vacuoles that became very heavily laden with the neutral red (Figs. 13 and 14). These cells, less numerous but much larger than the second type, are scattered in more or less irregular patches throughout the entire epithelial lining. When viewed with the binocular dissecting microscope, they stand out very conspicuously, especially the many large giant cells. The second type, smaller in size, has fewer and less deeply staining granules, arranged in a circle around a relatively large nucleus. Since the epithelial cells are on the surface, the neutral red has easy access and probably diffuses into the cells as rapidly as the granules and vacuoles form, and the slow increase during the first 3 or 4 hours probably is a fair indication of the rate at which the vacuoles and granules develop. The larger cells seem to develop vacuoles at a faster rate than the smaller ones and to reach a maximum earlier, maintaining this maximum for a considerable period of time. In large pieces of the bladder one can see the brilliantly stained epithelial surface in marked contrast to the pale muscular layers, for only after several hours does the latter accumulate enough red vacuoles to become conspicuous. This is due partly to the slowness of penetration by the dye. When the epithelial wall of the bladder is thrown into folds, as it usually is in the larger pieces, the neutral red appears first in the high spots of the folds, often long before the cells in the depths show any appreciable color. This is probably

merely a question of diffusion of the dye in the solution itself. Red granules and vacuoles were observed in the cells from 24 to 192 hours in series 1, from 12 minutes to 120 hours in series II, from 6 to 72 hours in series III, and from 1 to 3 hours in series IV. Macrophages are fairly abundant in the bladder wall and live longer than most of the other types of cells.

The *tracheal epithelium* was about as persistent as the cartilage but was not always present in the spreads. In series I, red granules and vacuoles were seen from 24 to 192 hours, in series II from 10 minutes to 120 hours, and in series III from 6 to 120 hours. In series IV the epithelium was observed at 1 and 6 hours; it probably lived longer, but unfortunately our spreads did not contain these cells at later periods. It was possible to watch with the low power the gradual accumulation of red color in the epithelial cells of large pieces, until finally the whole lining of the trachea was deep red. Ciliary movement was observed in the bronchi 120 hours after death in series I and in series III.

Observations on the *tongue ectoderm* were unsatisfactory. In series I, red granules were seen at 24, 48, and 192 hours but not at 72, 96, 120, 168, or 240 hours. In series II, red granules were seen in some of the cells at 45 minutes, 4 hours, and a very few at 48 hours, but none at 13, 18, 36, 96, 120, or 144 hours. In series III, granules were found at 48, 72, and 96 hours, but none at 12, 24, or 60 hours. In series IV, although observations were made from 1 to 24 hours after death, no red granules were seen in the cells. On the base of each papilla there developed a superficial area of pink granules or small vacuoles. These differed so markedly from the other vacuoles and granules that we have not considered them as indicators of the condition of the cells. Other superficial cells did not develop granules or vacuoles and they probably prevented the penetration of the neutral red to the deeper layers. At the edge of the cuts the cells were killed and the deeper layers of the epithelium were stained a diffuse pink. The granules and vacuoles occasionally seen in the cells of the deeper layers were observed only in spreads from exceptionally favorable pieces, and we doubt if our data give an adequate idea of the period of survival of these cells. Macrophages were very abundant in the connective-tissue spaces beneath the ectoderm and retained their red granules for 240 hours in series I, 72 hours in series II, 96 hours in series III, and 12 hours in series IV.

*Endothelium* was observed in the capillaries of various organs. The granules and vacuoles that take up the neutral red did not always appear to develop as rapidly as in most other types of cells. In series I, endothelial cells with red vacuoles were seen from 24 to 168 hours; in series II from 35 minutes to 72 hours; in series III, red vacuoles were seen at 12 hours; in series IV observations at 1 and 2 hours did not reveal red granules, but the same material kept for 24 hours longer in the neutral-red solution showed cells containing red vacuoles, indicating that they were alive at the time of the first observations. The size and number of vacuoles varied greatly in different spreads and in different parts of the same spread. The endothelial cells in some capillaries were filled with red vacuoles, whereas others were entirely without them (Figs. 16 and 17). At the first observation in series II there were a very few scattered vacuoles in the capillary walls, the number slowly increasing until the cells became quite full of them.

In the thymus, *small lymphocytes,* with from one to four small red vacuoles or granules, were seen from 24 to 168 hours in series I, from 25 minutes to 48 hours in series II, at 60 hours in series III, and at 1 and 3 hours in series IV. *Large lymphocytes,* containing a greater number of red granules and vacuoles, were seen at the same periods. The *microcytes,* with one or two rather large red vacuoles, were usually seen at the same time. Large macrophages were fairly abundant and usually survived as long or longer than the other cells. Similar cells from the spleen and lymph nodes were observed. They lived longer in the thymus than in the lymph nodes and longer in the lymph nodes than in the spleen. When the lymphocytes were dying, it was not uncommon to see cells, especially the large lymphocytes, with a few large vacuoles of a brownish red color and a dead nucleus having a sharp nuclear membrane.

In the lung, *epithelial cells,* with red granules and vacuoles, were seen from 24 to 144 hours in series I, from 35 minutes to 52 hours in series II, from 6 to 48 hours in series III, and from 1 to 3 hours in series IV. In series III there were possibly a few at 60 and 96 hours but none at 72 hours. The epithelial cells do not contain many vacuoles at any time and it was not always easy to determine whether the vacuoles were in these cells or in the endothelium. The macrophages, which occurred in great numbers, were the most characteristic objects in the lungs. They were found, loaded with red granules and vacuoles (Fig. 1), in series I from 24 to 240 hours, in series II from 30 minutes to 120 hours, in series III from 6 to 96 hours, and in series IV from 1 to 24 hours after death.

*Blood-cells* were examined in various organs and in blood clots. Leucocytes containing numerous red granules and small vacuoles were found from 24 to 120 hours in series I, from 45 minutes to 30 hours in series II, from 6 to 36 hours in series III, and at 1 hour in series IV. Red blood-corpuscles, with one to three or four granules or small vacuoles, were seen at 72 hours in series I, from 1 to 24 hours in series II, at 6 and at 24 hours in series III, and at 1 hour in series IV. Usually, very few if any of the corpuscles examined in a spread contained red vacuoles. Large macrophages were very abundant in

blood clots that had formed in the body cavities after the organs were removed for examination.

The *Kupffer cells* were usually seen without much difficulty and when first observed contained the maximum number of red vacuoles. They were often noticed in spreads in which the liver cells were dead or without vacuoles, and hence are apparently not killed as easily by the manipulations as the latter type. The vacuoles in the Kupffer cells were somewhat different in character from those formed in the liver cells, and they also either had a greater affinity for neutral red or were more easily penetrated by it. The fact that the Kupffer cells always contained the maximum number of vacuoles at the first observation indicates that the vacuoles are normal inclusions of these cells. They were seen from 24 to 120 hours in series I, from 20 minutes to 49 hours in series II, from 6 to 60 hours in series III, and from 1 to 12 hours in series IV.

*Brain macrophages* were observed in both the cortex and cerebellum (Fig. 21). They were not numerous and were usually scattered along the small blood-vessels to which they sometimes seemed to cling closely. They were often elongated, especially when attached to a blood-vessel. The vacuoles did not, as a rule, stain as deeply nor were they as numerous as those in the tissue macrophages. Like those in the tissue macrophages and in the Kupffer cells, they appeared to be different in character from the vacuoles formed in the tissue cells and are probably normal inclusions of these cells, as there was practically no increase in their number. The brain macrophages are not as large as the tissue macrophages. They were observed from 24 to 96 hours in series I, from 10 minutes to 48 hours in series II, from 6 to 96 hours in series III, and from 1 to 12 hours in series IV.

The *pancreatic gland cells* developed many small red granules that were always definitely located in the lumen end of the cell among the secretion granules. At the distal end of the cell there was usually a clear zone, free from red granules and secretion granules, which varied in width in different animals according, apparently, to the state of secretory activity as already noted in the cells of the salivary gland (Figs. 8 and 9). The red granules were observed from 24 to 96 hours in series I, from 30 minutes to 49 hours in series II, from 6 to 96 hours in series III, and at 3 hours in series IV. Macrophages were very rare.

*Liver cells* usually developed granules and vacuoles rather slowly and the cells on the surface, especially the uncut surface, showed the red color long before the more deeply placed ones. The liver cells are apparently easily killed and in many of the spreads no living ones, or at most only small patches of them, could be seen, whereas practically all the cells on the uninjured uncut surface of the larger pieces were filled with vacuoles. The vacuoles varied in size and were usually scattered through the cell (Figs. 10 and 11). Occasionally, red granules were seen only in that end of the cell bordering the bile capillary (Fig. 12). The vacuoles were found from 24 to 96 hours in series I, from 20 minutes to 15 hours in series II, and from 1 to 12 hours in series III. Although numerous observations were made in series IV, no cells containing red granules or vacuoles were seen. Further tests, with due appreciation of the susceptibility of the cells to mechanical injury, would probably have revealed living cells in series IV and for longer periods in series II. The large tissue macrophages are rare in the liver.

*Testis.*—The *Sertoli* cells were very conspicuous objects in spreads from pieces of the testis cut up in the neutral red solution. They formed regular patterns in the walls of the tubules (Fig. 20). The long axis of these cells is normally perpendicular to the surface of the tubule, but under pressure of the coverglass they became bent parallel to the surface, especially at the cut end of the tubule (Fig. 19), where they often became detached and floated off with a group of attached spermatogonia. Red vacuoles were observed in series I from 48 to 96 hours, in series II from 7 minutes to 16 hours, and in series IV at 3 hours after death. No observations were made in series III. It seems probable that the vacuoles are normally present in these cells. Macrophages were rarely seen. Interstitial cells with red granules and vacuoles were occasionally observed as early as 45 minutes in series II. Male germ cells and moving spermatozoa with one or more red granules were occasionally seen in series II from 40 minutes to 13 hours after death.

*Mesenchyme cells* were observed in various places but they did not often contain red granules or vacuoles. These cells are apparently rather sensitive; the nuclei were usually stained pink, indicating that the cells were dead. We have not attempted to distinguish between the various types of cells which constitute the connective tissues, except the tissue macrophages. It is rather surprising that the mesenchyme cells did not show up better. Red granules and vacuoles were observed in the cells from 24 to 72 hours in series I, from 17 minutes to 72 hours in series II, from 6 to 48 hours in series III, and at 3 hours in series IV. These cells did not develop as many vacuoles as some of the other types.

The *follicular cells* of the ovary were rather difficult to observe in spreads and we have comparatively few observations on the granules and vacuoles. Dense tissues like the ovary and adrenal are difficult to handle by our methods. Red vacuoles and granules were observed at 24 and at 72 hours in series I, from 24 to 30 hours in series II, at 12 and at 48 hours in series III, and at 1 hour in series IV. Observations on the *ova* were very difficult and unsatisfactory. In series III many fine granules were seen in the ovum at 12 hours but none at 24 or at 48 hours. In the other series we did not suc-

ceed in getting satisfactory observations. The *germinal epithelium* contained red granules and vacuoles from 3 to 5 hours in series II. No special attempt was made to observe it in the other series.

The *uterine epithelium* developed rather small granules and vacuoles. They were first seen 20 minutes after death in series II and gradually increased in number during the next 3 or 4 hours. Large areas of the epithelium observed at this time were deep red in color under the Greenough binocular, but quite different in character from the bladder or tracheal epithelium. In series I, granules and vacuoles were observed from 24 to 72 hours, in series II from 20 minutes to 53 hours, in series III from 6 to 12 hours, and in series IV at 1 hour. The *uterine gland cells* often became loaded with red granules and vacuoles which retained their color about as long as did those in the epithelial cells.

The *epithelial cells* lining the tubules of the epididymis contained red granules and vacuoles at 48 hours in series I, from 1 to 18 hours in series II, and at 3 hours in series IV. There were no observations in series III.

The *adrenal cells* are difficult to examine by this method; few of the essential ones containing red granules could be seen, in spite of many observations. In series I, many cells appeared to contain red granules at 24 hours but none were observed from 36 to 240 hours. In series II, cells were occasionally found with red granules from 15 minutes to 32 hours, and in series IV at 1 hour. None were seen in spreads from series III. Macrophages loaded with red were found in the capsule, but none in the body of the organ.

Very rarely were *fat cells* with red granules and vacuoles observed. In series II they were found at 4 to 18 hours. None were seen in series I and III. In series IV, fat-cells examined 1 hour after death were clear, but after standing for 24 hours in the neutral-red solution red vacuoles were found in some of them. They were not seen at any other time in this series. Large macrophages were abundant (Fig. 3).

*Epithelial cells* from the intestine showed red granules and vacuoles from 1 to 18 hours in series II, but not from 24 to 144 hours. None were seen in series I, III, or IV. The fact that the intestine is always infected probably explains why its cells did not live long. Macrophages were not often observed.

The *nerve cells* in the cortex and cerebellum rarely gave any indication of red granules and vacuoles. A few cells with pink vacuoles and granules were seen 15 minutes to one-half hour after death in series II. Most of the nerve cells at the periphery began to show a diffuse pink at this time, indicating that they were dead. An hour later practically all the cells were a diffuse pink in color in fresh spreads. The presence of brain macrophages and endothelium with red granules and vacuoles indicated that the environment was not especially unfriendly to life (Fig. 21). Death of nerve cells probably proceeds so rapidly that the granules and vacuoles do not have a chance to form.

Observations of *skeletal muscle* were made on the diaphragm, tongue, and abdominal muscles, but in no case were we able to find red granules or vacuoles in the fibers. Many observations were made in each series. Striated muscle behaved in a peculiar manner toward neutral red. The fibers rapidly became a diffuse deep pink, more marked than in other types of cells after death. We are unable to explain this diffuse coloring of muscle, as it is hardly to be expected that the fibers should die as rapidly as the diffuse coloration seemed to indicate. The contractile tissue evidently contains some diffuse substance that has a marked affinity for neutral red. Large macrophages, always abundant between the fibers and especially in the larger connective-tissue spaces between muscle bundles, were loaded with red granules and vacuoles.

*Heart muscle* behaved in a manner similar to skeletal muscle and always showed a diffuse pink. The large macrophages were not as common as in skeletal muscle, but they took up the neutral red in exactly the same manner.

A few scattered observations on the mesothelium of the body cavities, the epithelial cells of the choroid plexus (Fig. 23), the retina, iris, and cornea, showed that there is a similar accumulation of granules and vacuoles in these cells.

### Observations on Other Animals

Human tissues from three autopsies were examined in the usual manner as spreads from small pieces cut up in the neutral-red solution. Tissues from the first autopsy (the patient having died of carcinoma of the stomach) were examined 6 to 7 hours after death and, although the cadaver was in the ice-box at 37°F. for an hour during this time, only a few macrophages in the lungs and capsule of a lymph node and a few Kupffer cells contained red vacuoles. The other cells from the lung, lymph-node and liver, and all the cells of the testis, kidney, spleen, and skeletal muscle that were examined, were stained a diffuse pink by the neutral-red solution in which small pieces were cut up. In the second case (a child autopsied 31 hours after death) a few macrophages containing red vacuoles were found in the diaphragm, pancreas, intestine, capsule of a lymph-node, blood clot, and thymus. The body was in the ice box at 37°F. for 15 hours of this period. None of the other tissues examined, striated muscle, epididymis epithelium, adrenal, pancreas, liver, lymphocytes, tracheal cartilage, mesenchyme, endothelium, or smooth muscle, contained red vacuoles. Some of the tissues from the third autopsy (a 7½ months' fetus) were examined about 24 hours after death. The fetus had been kept in the ice-box for about 18 hours. Lung, diaphragm, kidney, pancreas, lymph node, liver,

spleen, trachea, thymus, adrenal, and retroperitoneal tissue were examined. Macrophages with red vacuoles were found in the lymph node, spleen, thymus, retroperitoneal tissue, and capsule of the adrenal, but no other types of cells containing red vacuoles were seen.

Cells from various organs of the rabbit were examined as in series II from 15 minutes to 5 hours after death. About the same accumulation of granules and vacuoles occurred as in the cells of the rat (Fig. 15). The lungs contained many large macrophages; the adrenal cells showed a more pronounced accumulation of red vacuoles than in the rat. A fetus 6.5 cm. long, from the same rabbit, was examined especially for the macrophages in the lungs, but none were found, although these cells were numerous in the subcutaneous tissue. The epithelium of the intestine and kidney contained many red vacuoles 5 hours after death.

Cells from various tissues of a guinea-pig were examined, as in series II, from 45 minutes to 30 hours after death. The accumulation of red vacuoles and granules presented very much the same picture as seen in the rat under similar conditions (Figs. 22 and 24). The macrophages were numerous in the lungs.

## DISCUSSION

Other methods might perhaps be employed which would preserve the life cells for longer periods, but these observations establish the fact that the different types of cells survive for different periods of time after death, and that the manner in which the tissues are treated makes a very great difference in the length of survival of all types of cells.

It is desirable to consider the nature of the granules and vacuoles and their probable origin before we attempt to explain why cells lived longer under some conditions than under others, and why certain cells always lived longer than others under the same conditions. With the exception of the macrophages, Kupffer cells, Sertoli cells, and leucocytes, probably none of the cells examined contained, under normal conditions in the body, more than a few, if any, granules and vacuoles having an affinity for neutral red. Such granules and vacuoles developed at different rates in the various types of cells, but we have not followed this particular phase with sufficient care to present here any definite data. Endothelium and smooth muscle often seemed to develop the vacuoles more slowly than most other types of cells. The number and size of the granules and vacuoles sooner or later attained a maximum state, which was different for each type and which was maintained for varying periods; then, as the cells approached the last phases of life, there was a diminution in the number of the red granules and vacuoles, until finally all the red color was lost. The color sometimes did not disappear from all the vacuoles until after the nuclear changes indicating the death of the nucleus had taken place. Increased refractivity and visibility, accompanied by the appearance of a marked nuclear membrane, were the most characteristic death changes in the nucleus. Dead nuclei were easily distinguishable from living ones and were very similar to those seen in cells after fixation, before dehydration and staining. The red color that sometimes lingered in the granules and vacuoles after nuclear death indicated that not all parts of the cell died at the same time; that the nucleus might have died before the cytoplasm and that some regions of the cytoplasm died before others.

The factors involved in the production of these granules and vacuoles is not altogether clear. There is, of course, first of all, the question whether they are to be regarded as food vacuoles, as an accumulation of waste products, or as autolytic products. It is possible, though not probable, that the condition of functional stress of the cells after the death of the animal might produce such alterations in the surface layer and in the body of the cytoplasm as would permit the rapid diffusion of dissolved food and other substances from the fluids still bathing the cells and their segregation as granules and vacuoles; to these something must be added from the cell itself to give to the resulting granules and vacuoles an affinity for neutral red. Such an explanation, however, does not seem very tenable. As the cells remained alive, they must have been undergoing metabolic processes, utilizing their own substance or substances in the fluid surrounding them; in either case the difficulty of eliminating waste products would seem to be obvious and so the granules and vacuoles might be looked upon as accumulated waste products. It is very doubtful if food substances surrounding the cells are present in sufficient quantity to play any rôle, in view of the greatly reduced supply of oxygen. So for the present, at least, it seems more reasonable to consider the granules and vacuoles as the waste products of autolytic or self-digestive processes and their affinity for neutral red as a coincidence. We can hardly regard them as a segregation apparatus, as Evans and Scott (1921) have interpreted somewhat similar vacuoles found in cells after the injection of acid-azo dyes into living animals; they are much more like the degeneration granules and vacuoles described by W. H. Lewis (1919) in the cells of tissue-cultures. If, then, they are to be considered, for the present at least, as the accumulated products of autolysis, *starvation* granules and vacuoles would be perhaps as appropriate a term as *degeneration* granules and vacuoles.

Mrs. M. R. Lewis has recently shown that a trace of ammonia added to the medium of tissue cultures produces a prompt formation of vacuoles which disappear if the cultures are subsequently washed with a fresh nutrient medium. This would appear to indicate that the vacuoles are produced by the accumulation of the

waste products of nitrogenous metabolism; in other words, that they are a sign of degeneration or autolysis produced by the breaking down of the cytoplasm and an accumulation of the resulting waste products.

Wells ('06) examined histologically from time to time various tissues undergoing autolysis, noting the relative rates of alteration, especially in the nucleus. The autolytic rate in such supposedly dead tissues did not correspond to the periods of survival as determined in our experiments. Brain cells, which we found to die rapidly, according to Wells retain their essential morphological character longer than most of the body cells.

In series IV, where the entire animal was kept at room temperature, the factors contributing to the rapid death of the cells would seem to be the temperature, the spread of infection from the alimentary canal, the absence of a pathway for the elimination of waste products, and the lack of oxygen and food. The first two factors would differentiate it from series III, where the entire animal was kept on ice at 37°F. There was a very great difference in the length of survival of the cells of the two series, as can be seen readily in the table. Temperature alone is not so directly important in producing rapid death, since in series II the cells in the small fragments which were kept at room temperature lived as long on the average as when the whole rat was kept in the ice-box at 37°F. (series III). Infection may play a very important rôle, for the intestinal cells rarely lived more than a few hours in any of the series and often no living cells with vacuoles were seen at any time.

In series II (small fragments of tissue kept at room temperature in the neutral-red solution) and III (the entire animal kept at 37°F.) the survival period was approximately the same. The retardation of metabolic processes in series III was counterbalanced by more rapid metabolic processes with increased opportunity for the elimination of waste by diffusion. Infection was not entirely eliminated in series II, as only part of the tests were made under strictly aseptic conditions.

In series I infection was practically eliminated except for a few of the organs. Metabolism was retarded by the cold and the few cells that survived beyond the period for series II and III were probably favorably located.

We are hardly in a position to go farther into an explanation of the causes which bring about cell death under the conditions of our experiments. They are undoubtedly closely related to the factors which produce the granules and vacuoles. Most of the cells were probably going down hill from the hour the animal was killed.

The various types of cells must differ from each other in many ways, in chemical and physical constitution, in the rate of metabolism, in the amount of oxygen, various salts, and foodstuffs used, and in the chemical composition of the waste products. Each cell is a complex mechanism which acts effectively only in the normal environment in the animal and this environment varies more or less for each type. When the animal dies the environment is changed, whether the cells are left in the body or the tissues are removed and placed in the neutral-red solution. The period of survival in the changed environment depends on how well each type of cell is naturally fitted to live there. Now, when we consider the natural environment together with the known function of each type, we can perhaps see how this corresponds in a way with the varying periods of survival.

The tissue macrophages may owe their ability to survive for relatively long periods to the fact that they are constructed to live, under normal conditions, in the tissue spaces without close contact to either oxygen supply or special elimination paths, and that they normally contain vacuoles which are probably, in part at least, composed of waste products.

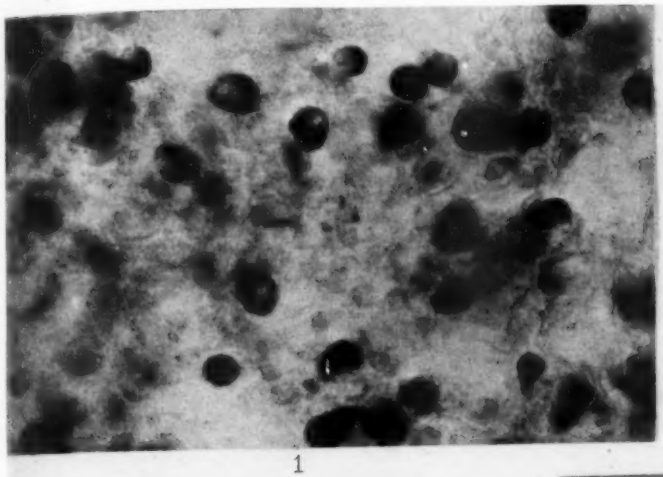
The cartilage cells likewise, though fixed in position, are naturally rather far removed from the capillaries, so that neither of these types of cells would respond to the cutting off of the blood supply as quickly as cells that have perhaps a higher rate of metabolism. Although the kidney cells are accustomed to a rich blood supply, they are also constructed to deal with waste products, so that the accumulation of waste products in the form of granules and vacuoles within them is perhaps not so detrimental as to other types of cells.

The epithelial cells from the bladder, tongue, and trachea might also be expected to survive for a relatively long period, since their peculiar location on the surfaces would indicate that they were adapted for living some distance from the blood supply.

The list of cells given in the table might thus be followed in the order of their survival and reasons suggested for their relative length of survival, without, however, bringing us much nearer to the actual reasons, which are still obscure. Since in each series the environment is approximately the same for all cells, the differences in length of survival must depend on the make-up of the cells themselves.

## Summary

Small pieces of tissue from various organs of the rat, examined at varying periods after death, showed that most types of cells survived for a time and certain types of cells survived longer than others, in approximately the following order: tissue macrophages, cartilage cells, kidney-tubule cells, smooth-muscle cells, salivary-gland cells, bladder epithelium, tracheal epithelium, tongue epithelium, endothelium, lymphocytes, large lymphocytes, micro-



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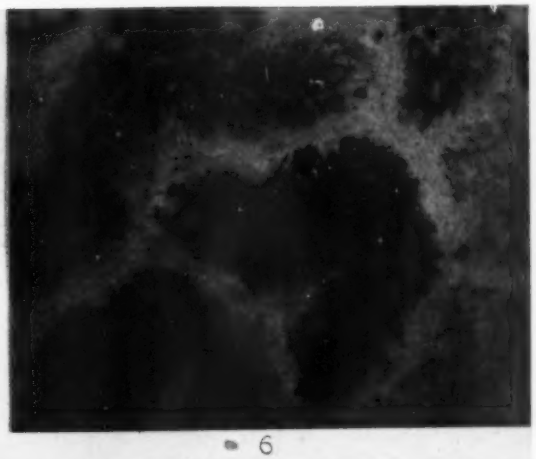

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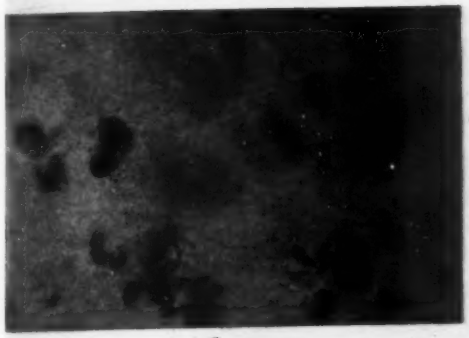

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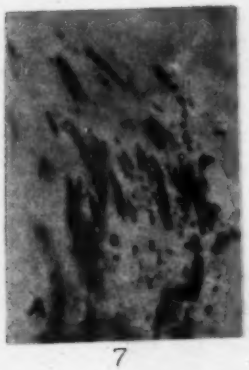

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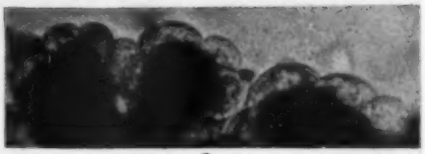

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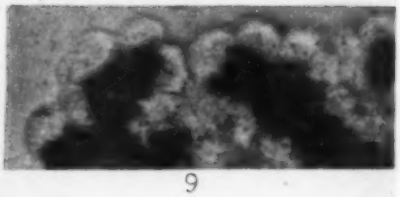

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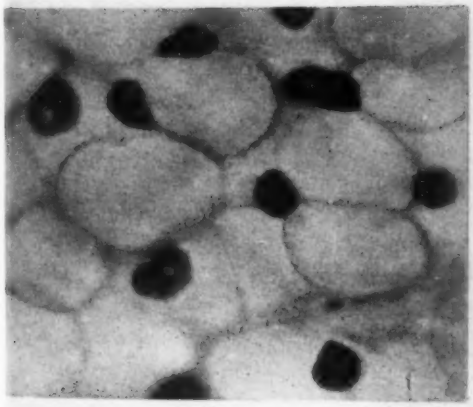

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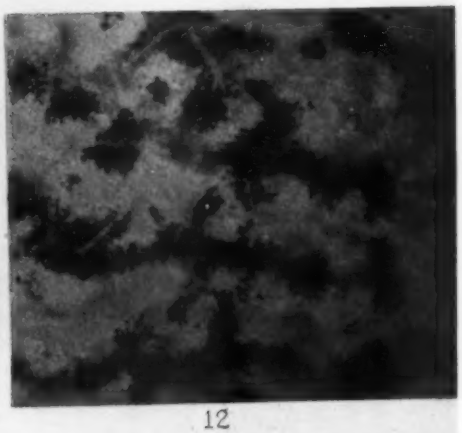

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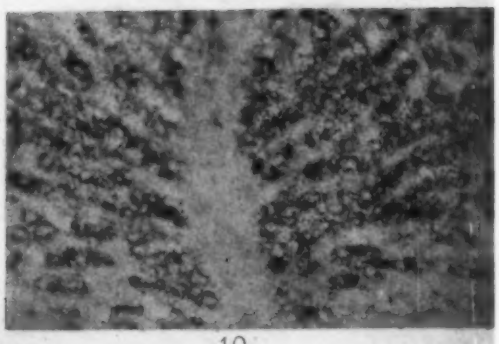

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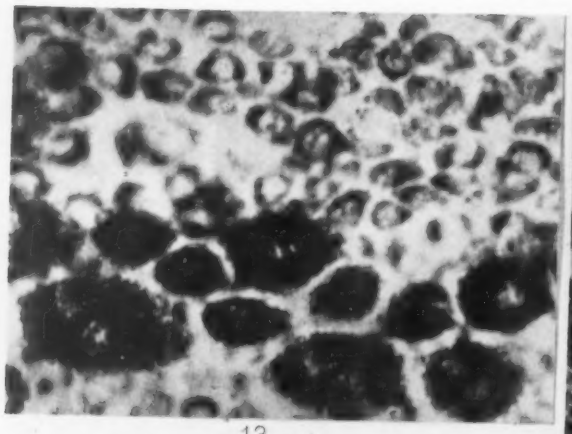

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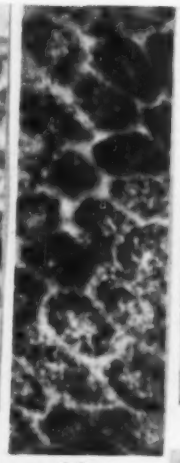

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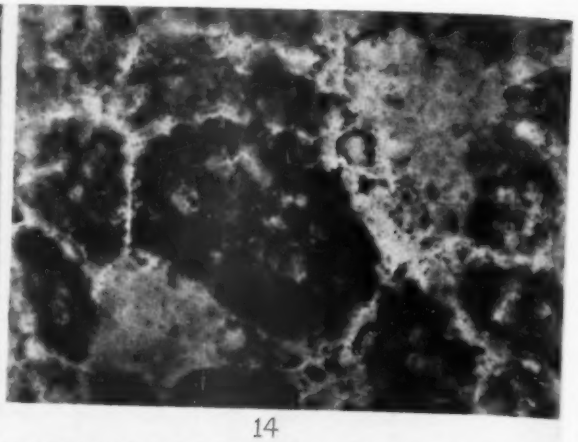

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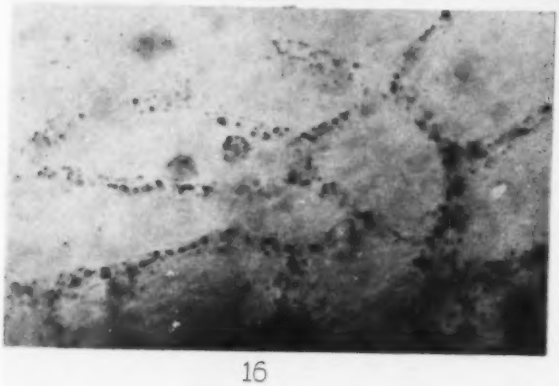

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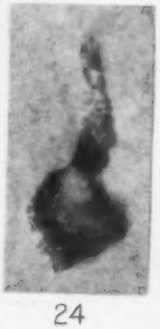

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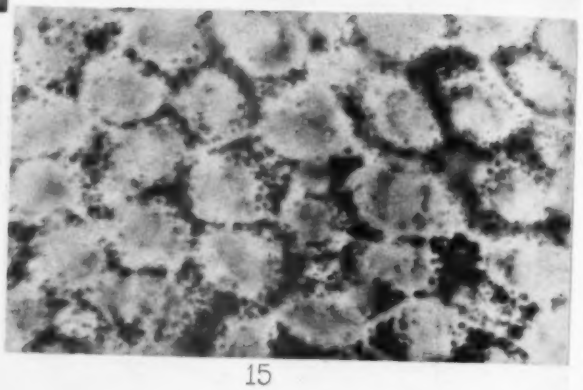

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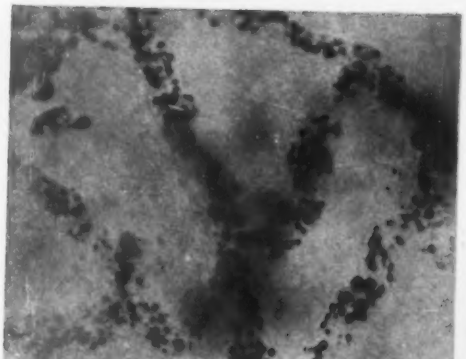

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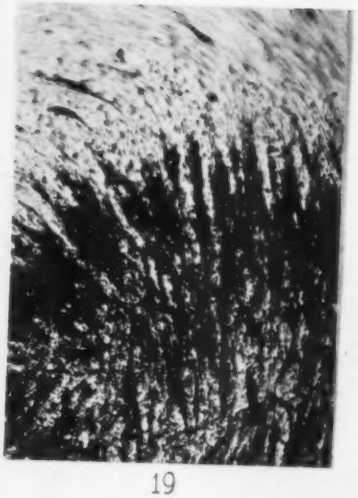

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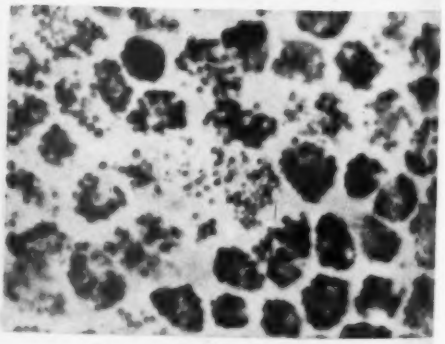

22

cytes, lung epithelium, leucocytes, Kupffer cells, brain macrophages, pancreas cells, red blood-corpuscles, liver cells, Sertoli cells, mesenchyme, ovarian follicular cells, epididymis epithelium, adrenal cells, intestinal epithelium, and nerve cells. Macrophages survived for about 240 hours; brain cells for less than an hour; striated muscle developed no granules or vacuoles.

The criteria for life were: (1) the presence in cells of granules and vacuoles that had an affinity for neutral red while the cells were alive; almost all types of cells either had such granules or developed them after the death of the animal; (2) a homogeneous nucleus without trace of a nuclear membrane. The criteria for death were: (1) loss of color from the granules and vacuoles; (2) diffuse pink staining of the cytoplasm and nucleus; (3) the appearance of a sharp and distinct nuclear membrane and a change in the texture of the cytoplasm and nucleus.

Four series of experiments were conducted: In series I organs were kept at 37°F.; in series II tissues were cut up into minute fragments in a neutral-red solution and kept at room temperature; in series III the entire animal was kept at 37°F.; and in series IV the dead rat was kept at room temperature. Cells lived longest in series I, about half as long in series II and III, and a very much shorter time in series IV.

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## LEGENDS FOR FIGURES

FIG. 1.—Macrophages in the lung heavily laden with neutral red granules and vacuoles. Series II, 5 hours after death. X 480.

FIG. 2.—Macrophages in the lung; much of the red color has gone. Series I, 192 hours after death. X 480.

FIG. 3.—Macrophages in fat. Series I, 36 hours after death. X 480.

FIG. 4.—Macrophages in renal capsule. Series II, 51 hours after death. X 146.

FIG. 5.—Tracheal cartilage. Granules and vacuoles near cell nuclei. Series I, 72 hours after death. X 480.

FIG. 6.—Kidney tubules. Most of the tubule cells have large irregular vacuoles. Series II, 6 hours after death. X 480.

FIG. 7.—Smooth muscle from bladder. Note linear arrangement of vacuoles. Series III, 37 hours after death; ice box 12 hours, room temperature 25 hours. X 480.

FIG. 8.—Pancreatic gland. Series I, 5 hours after death. X 480.

FIG. 9.—Pancreas. Series III; ice box 37°F. 12 hours after death. X 480.

FIG. 10.—Liver cells. Series I, 72 hours after death. X 146.

FIG. 11.—Liver cells with vacuoles. Series I, 72 hours after death. X 480.

FIG. 12.—Liver cells. Series II, 5 hours after death. X 480.

FIG. 13.—Bladder epithelium, small mononuclear, binucleate and giant cells with vacuoles. Series III, 37 hours after death; ice box 12 hours and room temperature 25 hours. X 480.

FIG. 14.—Bladder epithelial giant cells. Series II, 4 hours after death. X 480.

FIG. 15.—Bladder epithelium from rabbit, examined as in series II, 5½ hours after death. X 480.

FIG. 16.—Capillary net from ovary, vacuoles in endothelial cells. Series I, 36 hours after death. X 480.

FIG. 17.—Capillary net from bladder, endothelial cells with large vacuoles. Series II, 48 hours after death. X 480.

FIG. 18.—Salivary gland cells crowded with vacuoles. Series I, 24 hours after death. X 480.

FIG. 19.—Sertoli cells in testis tubule under pressure. Series II, 4½ hours after death. X 480.

FIG. 20.—Sertoli cells in testis tubule. Series II, 16 hours after death. X 480.

FIG. 21.—Brain macrophages. Series I, 72 hours after death. X 480.

FIG. 22.—Peritoneum from diaphragm of guinea-pig examined as in series II, 26 hours after death. X 480.

FIG. 23.—Epithelial cells of choroid plexus. Series II, 4½ hours after death. X 480.

FIG. 24.—Macrophage from lung of guinea-pig examined as in series II, 23 hours after death. X 1450.

# A STUDY OF THE AUSTIN-STILLMAN-VAN SLYKE INDEX OF THE UREA EXCRETION

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and

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It is not within the scope of this short paper to attempt any review of the literature on urea excretion, and it is with considerable trepidation that one ventures to add to its bulk. Since the original work of Ambard in 1909, much evidence, clinical and experimental, has been accumulated to demonstrate that the rate of urea excretion may be used as a rough indicator of the functional capacity of the kidneys, although the mathematical formulations of the "laws" governing this rate have varied from time to time and from person to person. A detailed discussion of the subject is presented in the recent paper of Austin, Stillman, and Van Slyke, to which the reader is referred.[1] Their formulation of the relationships of the factors involved is expressed as $K=\frac{D}{BV\sqrt{W}}$; in which D is the total urea excretion in grams for 24 hours, V is the urine volume in liters for 24 hours, B is the concentration of the blood urea in grams per liter, W is the patient's weight in kilos, and K a constant which in normal individuals has been found to vary between the limits 4.5 and 10.5. The data upon which their calculations are based were derived from repeated observations upon a few healthy subjects. It is naturally a matter of no little interest to study the behavior of the Austin-Stillman-Van Slyke constant under varying conditions of disease.

The subjects, all of whom were males, were chosen more or less at random from the public wards of the Johns Hopkins Hospital. With one exception, one observation only was made on each patient. The urine was collected usually for a period of seventy minutes; the blood for urea determination was drawn as nearly as possible at the middle of the period. There was no preceding starvation of the patients, although they were requested to abstain from taking anything by mouth for the duration of the test. In each instance, furthermore, the morning meal preceded the first voiding by at least two hours. The blood urea was determined by the Van Slyke-Cullen method. Urea of the urine was determined by the Van Slyke-Cullen modification of Marshall's urease method.[2] The results are presented in tabular form below.

## Discussion

Little comment is necessary upon Cases 1 to 9, with the exception of No. 6. Here definite urinary findings and a decreased phenolsulphonephthalein excretion are associated with a normal index. However, the urine volume and the total urea excretion are certainly excessive, particularly as there was no polyuria, and it is reasonable to presume that the patient's slight prostatic hypertrophy interfered with the complete emptying of the bladder at the first voiding.

It is noteworthy that two cases of myocardial failure, in each of which there was evidence of renal congestion, yield indices well within the normal range. In each case, observations were made before complete recovery of compensation had occurred. In neither case was there final evidence of permanent renal damage, as judged by the usual criteria.

Case 10 was that of a patient suffering from lobar pneumonia, in cultures from whose sputa a type I pneumococcus had been recovered in large numbers. Following the therapeutic administration of 700 cc. of homologous immune serum, a well-marked urticarial rash developed, and coincidently urinalysis demonstrated marked renal irritation. In the face of this, a normal urea excretory index was found. This result is of interest when compared with the observations of Rackemann, Longcope, and Peters on the chloride and water excretion in serum sickness.[3]

Two cases of pernicious anemia yielded definitely low results, while a third gave a low normal. Case 15 is of particular interest. The first estimation was made during a period of comparative quiescence, in which the red cell count had remained practically stationary for two weeks at a level just below 2,000,000, and the hemoglobin had been at 45% (Sahli). On the day of the determination, the actual count was 1,804,000, and the hemoglobin 43%. Shortly thereafter an active relapse ensued, the patient's red cell count dropping from 2,200,000 to 1,200,000 in seven days. On the fourth day of this relapse two further determinations were made, the second following the ingestion of a high nitrogen test meal, which had been given for quite another purpose. It is of great interest to note that the coefficient, which had stood at 4.33 during the remission, dropped to 3.55 during the course of the relapse, and reached the still lower level of 3.07 following the ingestion of a meal very rich in protein. The actual red cell count on the day of this experiment

| Name | Clinical Diagnosis | Age | Urine Picture | B. P. Range | Eye Grounds | Arterio-sclerosis | Size of Heart (cm.) Rt. | Lt. | Phthalein | NPN W'r mg. 100 c.c. | kilos. | Blood Urea mg. 100 c.c. | Urine Vol. 70 m. 24 hrs. Liters | | Urine Urea 70 m. 24 hrs. Grams | | Index | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1—Chalk | Pneumonia Convalescent | 33 | Sp. gr. 1010-1028 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 100/75-90/55 | Negative | Slight | 2.5 | 9 | 2 hours = 60 | 30.1 | 64 | 16.6 | 0.3 | 6.0 | 1.124 | 22.48 | 6.99 | |
| 2—Applebaum | Arterio-sclerosis | 65 | Sp. gr. 1015-1030 Casts 0. W.B.C. 0. Blood 0. Alb. trace | 140/72 | Vascular changes | Extreme | 2 | 9 | 18+10 = 28 | 46.2 | 86.5 | 24.4 | 0.053 | 1.06 | 0.7925 | 15.85 | 8.39 | |
| 3—Keith | Tabes dorsalis | 42 | Sp. gr. 1018-1025 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 165/90-117/84 | Negative | Slight | 2.5 | 8.5 | | 34.3 | 59 | 14.8 | 0.077 | 1.54 | 0.4898 | 9.796 | 6.94 | |
| 4—Talkington | Neurasthenia | 43 | Sp. gr. 1018 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 118/82 | Negative | None | 3 | 8.5 | | 32.9 | 60.5 | 15.6 | 0.045 | 0.9 | 0.49156 | 9.831 | 8.54 | |
| 5—Sokoski | Emphysema Myocardial insufficiency | 57 | Sp. gr. 1016-1040 Casts 0. W.B.C. 0. Blood 0. Alb. + | 150/90-100/57 | Negative | Moderate | 5 | 9 | 65+20 = 85 | 40.0 | 69.5 | 25.2 | 0.05 | 1.0 | 0.70661 | 14.132 | 6.73 | |
| 6—Henderson | Elephantiasis Chronic nephritis | 56 | Sp. gr. 1010-1016 Casts + W.B.C. + Blood + Alb. trace | 120/66 | Negative | Slight | 3.5 | 13 | 30+5 = 35 | 36.2 | 103 | 14.0 | 0.18 | 3.6 | 1.10 | 22.0 | 6.17 | |
| 7—Block | Sub-acute endo-carditis. Strepto-coccus viridans | 45 | Sp. gr. 1018-1024 Casts 0. W.B.C. 0. Blood once. Alb. 0. | 128/75-100/57 | Vascular changes | Moderate | 3 | 10.5 | 38+15 = 53 | 36.4 | 52 | 21.5 | 0.095 | 1.9 | 0.5355 | 10.71 | 5.02 | |
| 8—Lesille | Peritoneal carcino-matosis | 58 | Sp. gr. 1018-1021 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 190/95 | Negative | Moderate | 3.5 | 12 | 35+18 = 53 | 33.0 | 69.5 | 15.3 | 0.028 | 0.56 | 0.239 | 4.78 | 5.01 | |
| 9—Mitchell | Mitral stenosis Myocardial insuf. Chr. bronchitis | 36 | Sp. gr. 1018-1040 Casts 0. W.B.C. 0. Blood 0. Alb. trace | 115/75-90/65 | Negative | None | 4 | 9.5 | Unsuccessful | 33.3 | 70.5 | 14.3 | 0.095 | 1.9 | 0.489 | 9.78 | 5.91 | |
| 10—Erdman | Lobar pneumonia Serum sickness | 44 | Sp. gr. 1010-1020 Casts + W.B.C. + Blood + Alb. + | 124/75-90/60 | Negative | None | 3 | 10.5 | 40+? | | 68 | 11.9 | *(80m)* 0.258 | 4.644 | *(80m)* 0.646 | 11.628 | 5.50 | |
| 11—Groeninger | Chr. bronchitis Bronchial asthma | 51 | Sp. gr. 1026 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 142/50 | Negative | Moderate | 2.5 | 9.5 | 25+10 = 35 | 33.3 | 49 | 16.4 | 0.238 | 4.76 | 0.894 | 17.88 | 7.16 | |
| 12—Cosgrove | Diabetes insipidus | 52 | Sp. gr. 1003-1009 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 175/95-149/80 | Negative | Slight | 3 | 10 | 35+35 = 70 | 28.6 | 73 | 11.5 | 0.23 | 4.6 | 0.44642 | 8.928 | 4.24 | |
| 13—Heinicke | Pernicious anæmia | 41 | Sp. gr. 1011 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 114/64 | Negative | None | 4 | 9.5 | 35+20 = 55 | 23.3 | 73 | 16.7 | *(80m)* 0.109 | 1.962 | *(80m)* 0.528 | 9.504 | 4.74 | Stationary low count. |
| 14—Gibbons | Pernicious anæmia | 47 | Sp. gr. 1015-1026 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 90/50 | Negative | Slight | 3.5 | 9 | 45+20 = 65 | 33.3 | 66.5 | 15.1 | 0.0355 | 0.71 | 0.1975 | 3.95 | 3.80 | Stationary fairly low count. |
| 15—Lucas | Pernicious anæmia | 36 | Sp. gr. 1010-1018 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 110/50 | Negative | None | 3 | 8 | 2 hours = 30 | 36.2 | 59.5<br>60<br>60 | 18.2<br>21.1<br>*(60m)*<br>17.5 | 0.122<br>0.212<br>0.248 | 2.44<br>4.24<br>5.95 | 0.474<br>0.597<br>*(60m)*<br>0.422 | 9.48<br>11.94<br>10.128 | 4.33<br>3.55<br>3.07 | Stationary active relapse. |
| 16—Wilson | Chronic cardio-renal disease. Indolent ulcers | 53 | Sp. gr. 1015-1030 Casts 0. W.B.C. few Blood 0. Alb. trace | 190/115-140/85 | Slight vascular changes | Moderate | 3 | 13.5 | 38+15 = 53 | 38.7 | 75.5 | 24.7 | 0.05 | 1.0 | 0.4068 | 8.136 | 3.79 | |
| 17—Scheminant | Chronic nephritis | 30 | Sp. gr. 1010-1020 Casts ++ W.B.C. 0. Blood 0. Alb. ++ | 135/90 | Negative | None | 3 | 8.5 | 22+18 = 40 | 42.8 | 45.5 | 30.7 | 0.031 | 0.62 | 0.29365 | 5.873 | 3.60 | |
| 18—Stain | Hypertension Duodenal ulcer | 35 | Sp. gr. 1018-1030 Casts 0. W.B.C. 0. R.B.C. 0. Alb. trace | 200/135-160/110 | Slight vascular changes | Moderate | 3 | 12 | 20+40 = 60 | 39.8 | 54 | 19.9 | 0.076 | 1.52 | 0.26251 | 5.24 | 2.90 | |
| 19—Wickers | Hypertension | 37 | Sp. gr. 1008-1024 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 180/124-130/94 | Negative | Moderate | 2.5 | 8 | 10+35 = 45 | 40.6 | 61 | 16.8 | 0.244 | 4.88 | 0.2918 | 5.836 | 2.02 | |
| 20—Stollar | Chronic hypertrophic arthritis. Arteriosclerosis | 65 | Sp. gr. 1020 Casts 0. W.B.C. 0. Blood 0. Alb. 0. | 180/105-138/78 | Negative | Marked | 2.5 | 10 | 2 hours = 28 | 26.7 | 74 | 12.4 | 0.086 | 1.72 | 0.1745 | 3.49 | 2.49 | |
| 21—Hyason | Chronic cardio-vascular-renal disease | 53 | Sp. gr. 1012-1030 Casts ++ W.B.C. + Blood 0. Alb. +++ | 235/136-208/78 | Retinal arterioscleros-sis. Exudate. Few hæmorrhages. | Marked | 5 | 16 | 21+14 = 35 | 34.3 | 59 | 19.3 | 0.062 | 1.34 | 0.1894 | 3.788 | 2.21 | |
| 22—Levy | Arteriosclerosis. Arteriosclerotic ulcers | 70 | Sp. gr. 1022-1036 Casts occ. W.B.C. occ. Blood 0. Alb. 0. | 155/78 | Vascular changes | Extreme | 3.5 | 10.5 | 14+18 = 32 | 29.5 | 72.5 | 17.8 | 0.042 | 0.84 | 0.2875 | 5.75 | 4.14 | |
| 23—Stewart | Chronic nephritis | 43 | Sp. gr. 1010-1018 Casts 0. W.B.C. 0. Blood 0. Alb. ++ | 165/105-158/98 | Negative | Slight | 3 | 12.5 | 43+18 = 61 | 44.5 | 70 | 18.1 | 0.148 | 2.96 | 0.4855 | 9.71 | 3.73 | |
| 24—Metz | Sub-acute nephritis. Death | 32 | Sp. gr. 1029 Casts +++ W.B.C. ++ Blood ++ Alb. ++++ | 170/90-178/86 | Minimal retinal edema | None | 3.5 | 9.5 | Traces | 341.0 | 79 | 150.0 | 0.005 | 0.1 | 0.0041 | 0.082 | 0.02 | Dr. Darner* |
| 25—Webb | Sub-acute nephritis | 18 | Sp. gr. 1016-1021 Casts ++ W.B.C. + Blood + Alb. +++ | 140/78-107/50 | Negligible | None | 2.5 | 8 | Traces | 95.3 | 28 | 59.9 | 0.0265 | 0.53 | 0.0762 | 1.524 | 0.66 | |
| 26—Bazzle | Chr. cardio-renal disease. Broncho-pneumonia. Death | 52 | Sp. gr. 1015 Casts + W.B.C. + Blood + Alb. ++ | 150/80-117/70 | Retinal arterioscleros-sis. Massive exudate. Many hæmorrhages. | Extreme | 3.5 | 12.5 | Traces | 95.2 | 55 | 59.0 | 0.076 | 1.52 | 0.428 | 8.56 | 1.59 | Dr. Oppen-heimer** |
| 27—Leahy | Cholelithiasis. Obstructive jaundice | 61 | Sp. gr. 1020 Casts + W.B.C. + Blood 0. Alb. trace | 195/98 | Negative | None | 3.5 | 10.5 | 30+5 = 35 | 35.7 | 90 | 21.1 | 0.011 | 0.22 | 0.0379 | 0.758 | 0.81 | |

* Necropsy:—Acute and chr. diffuse nephritis. Anasarca. Ascites. Bilateral hydrothorax. Pulmonary edema. Broncho-pneumonia. Acute splenic tumor. Scarred rt. apex. Caseous mediastinal glands. Phleboliths of spleen and liver.

** Necropsy:—Chronic diffuse nephritis; arteriosclerosis; erosion and calcification of aortic valve with insufficiency and stenosis. Cardiac hypertrophy and dilatation. Acute bronchitis. Confluent lobular pneumonia. Acute splenic tumor. "Milk plaques" on heart. Old pleuritic adhesions.

was 1,780,000 and the hemoglobin reading was 37% (Sahli). Evidence of impaired renal function in severe anemias has been advanced by Mosenthal,[4] and Christian has presented observations suggesting the same conclusion in a series of cases of pernicious anemia.[5] However, as in Case 13, with a red cell count of 1,200,000, and a hemoglobin reading 25%, an index of 4.74 was found, the possibility of the participation of factors other than the severe anemia alone must be considered. This latter was a case in which the count had hovered just above one million for two weeks, with corresponding slight fluctuations in the hemoglobin readings. Case 14 at the time of observation presented a red cell count of 2,200,000, with hemoglobin of 38%, and was slowly losing ground. Coincidently with the gradual decline in the count, a definitely low index was found. The inference is attractive that during the active relapses of pernicious anemia there is a definite impairment in the function of urea excretion, which shows definite improvement during the remissions of the disease, and that perhaps this impairment is not dependent solely upon the severity of the anemia.

The early detection of failing kidney function in hypertension is an absorbing problem. It is noteworthy in that connection that Cases 18 and 19, who present little evidence of functional impairment by the usual tests, yield indices comparable to those obtained from outspoken cases of chronic nephritis.

Except for Case 27, little comment upon the remainder is necessary. Even cursory scrutiny of the data will suffice to demonstrate the constancy with which marked renal damage is associated with a lowered excretory index. In the case of obstructive jaundice, there is definite evidence of renal insufficiency, but the calculated coefficient is so low as to leave the impression that the collection of specimens was faulty. Yet a liberal allowance for error still gives a low index upon re-calculation, and it seems quite likely that in this case also there was a grave degree of kidney damage.


### Summary

Twenty-seven cases are presented in which the Austin-Stillman-Van Slyke urea excretory coefficient was determined. In borderline conditions, as well as in cases presenting marked evidence of renal damage, and also during the relapses of pernicious anemia, there was found a definite lowering of the value of the coefficient.


### Conclusions

The Austin-Stillman-Van Slyke coefficient of urea excretion may be of value in demonstrating renal insufficiency, and, particularly in borderline cases, deserves further study.

The authors desire to acknowledge their indebtedness to Dr. William S. McCann for helpful interest and valuable suggestions.

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Fig. 2.—Camera-lucida drawing of a cell containing pigment.

# STUDIES ON EXPERIMENTAL RICKETS. XXII.

## CONDITIONS WHICH MUST BE FULFILLED IN PREPARING ANIMALS FOR TESTING THE ANTI-RACHITIC EFFECT OF INDIVIDUAL FOODSTUFFS


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During the past two years we have carried out a study of the effects of faulty diets of various types on bone development. The results of this study have in part been reported in earlier papers.[1] Our observations have indicated that three dietary components are especially concerned in influencing the behavior of the several anatomic elements in the bones. These are calcium and phosphorus, and an organic substance which has as yet not been definitely characterized. We have recently demonstrated that this substance is not identical with fat-soluble A. The relative proportions of these factors determine whether the animal will develop bones which are normal or bones which are pathological; whether the animal will have rickets, or osteoporosis, or an osteosclerosis.

Mellanby and we ourselves have shown that an animal must grow in order to have rickets. Given a diet which induces rickets, the more rapidly the animals grow the

more extensive are the lesions which are characteristic of the disease. When growth is but slight the rachitic metaphysis will be narrow, and the other characteristics of rickets will be poorly developed. This we have described in the eighth paper of this series.[2] The diet must be excellent except as regards calcium, phosphorus and the calcium-depositing substance, in order that maximal growth may be secured. The proteins of the food mixture should be of good quality. There should be a moderate amount of fat-soluble A (anti-xerophthalmic substance) available, and the diet should contain a satisfactory amount of water-soluble B, and the inorganic elements other than calcium and phosphorus. The calcium-depositing substance should be distinctly limited. Furthermore, as we ourselves, and Sherman and Pappenheimer,[3] have shown, the quantitative relations between the calcium and phosphorus in the food supply is, within certain limits of concentration, of very great significance in influencing the development of the bones. The present paper deals especially with the effects produced on the anatomic structure of the bones by different ratios between the calcium and phosphate in the diet, other factors remaining essentially constant.

In a preceding paper of this series[4] we described in detail a method for preparing young rats for the demonstration of the anti-rachitic effect of various substances in the diet.

In order to make this test in a satisfactory manner we have found it necessary to have the diet on which the animals are fed in the preparatory period of 25-35 days very nicely adjusted with respect to several factors. It is our purpose in the present communication to define more clearly the dietary conditions under which this test can be satisfactorily carried out, and to describe the lesions which are likely to be observed provided the composition of the diet used to prepare the animals for the test deviates, even to a relatively slight degree, from those which we have laid down.

Table I shows the composition of the diets discussed in this paper, together with their sources of additional calcium and phosphorus. In only two diets was the amount of phosphorus increased over that furnished by the organic components of the food mixtures. The table also shows the absolute amounts of calcium and of phosphorus in the diets, and the atomic and weight ratios between these elements. The effects of the several diets on the histological structure of the bones of young rats which were confined to them were characteristic. A description of the bones of animals confined to this series of diets makes clear how sensitive the rat is to changes in the composition of its diet of a magnitude which would have been considered as of no significance a few months ago. This series of experiments makes it clear that extremely careful work is required in studies on bone growth. A full appreciation of this fact will go far toward preventing faulty interpretations by investigators who may undertake unsuccessfully to duplicate or extend observations in this direction.

### METHODS OF SELECTING AND PREPARING FOODSTUFFS

There are several details of importance in experimental studies in nutrition which, it appears, some experimenters are likely not to appreciate, which we take this opportunity to emphasize. These relate especially to (1) the selection of vegetable food substances for feeding purposes; (2) the purification of proteins to be employed in supplementing the proteins from natural foods, or for supplying the total protein moiety in diets consisting essentially of purified food substances; (3) the determination of the composition of the mineral salts to be employed in feeding experiments; (4) the proper preparation of fats for supplying in experimental diets the desired amount of fat-soluble A; and (5) the proper evaluation of the vitamin content of certain extracts of natural foods employed as additions to food mixtures for experimental purposes.

1. We have always been very careful to select for feeding purposes only such grains as are free from disease. Any one familiar with seed grains knows that it is frequently found that the germinating power of grain is low or lacking. There are a number of causes for this, the chief ones being damage to the grains by unfavorable climatic conditions during the last stages of development, and infection of the kernels by bacteria or moulds. An inspection of many samples of maize, for example, will show that the germ or "heart" is darkened, owing to invasion by bacteria. In such seeds extensive decomposition of the food substances may have taken place, and even toxic products may have been formed. In other grains, badly developed kernels with low germinating power are the rule rather than the exception. While in the Wisconsin Experiment Station, it was easy to select grains which were sound. Since transferring our work to Baltimore we have for the above reasons secured our grains only from reputable seed dealers who handle grains which are known to be healthy and to have the power to germinate. Grains for nutritive studies should never be purchased from feed stores, since they will in most instances be inferior, and to some extent contaminated with other seeds, and great variation of quality is to be expected.

The history of leafy structures which are to be used for experimental purposes should be known, and they should be practically free from stems. They should not have been cured in the field unless the weather was exceptionally favorable for rapid desiccation. Leaching and the growth of bacteria and moulds must be avoided in their preparation. It is well known that sufficient salts leach from shocks of grains standing in the field to fertilize the soil to an extent that may greatly stimulate the growth of the next year's crop on these spots.

2. In the purification of casein two methods have been almost universally employed, and it seems to us that considerable confusion is likely to arise unless certain standards are adopted in the preparation of food materials. This is especially true in the study of experimental rickets. It is well known that when casein is precipitated from milk it takes with it very considerable amounts of inorganic salts. Whey, which is formed in cheese making through the action of rennet, is very poor in calcium, since this element is in the main carried down with the curd. It is not possible by any simple and easy method to purify casein in considerable amounts so as to make it suitable for experimental purposes.

During the last few years it has become a matter of some importance to use casein for feeding experiments which was free from vitamins, and a number of investigators have subjected casein, the purity of which was not stated, to continuous extrac-

tion with hot alcohol for the purpose of freeing it from fat-soluble A and water-soluble B. One is led by the comments of certain workers to conclude that they are of the opinion that exhaustion with alcohol is the most important treatment to which casein should be subjected in order to make a preparation of high quality for feeding purposes. It is difficult to see how this idea gained credence. Dry casein is impervious to alcohol, and extraction with alcohol is not likely to be effective in removing impurities incorporated within the casein granules. It is next to impossible to grind casein to an impalpable powder, and even if it is so ground, it is difficult to extract it effectively in a continuous extractor, because the material packs so tightly that the solvent does not permeate it uniformly, but finds paths of little resistance and tends to follow them, leaving much of the material incompletely acted upon. It is not to be expected that inorganic salts will be removed to any great extent by alcohol, so a casein preparation may be very laboriously extracted, and yet be little more suitable for use in nutrition experiments than before it was treated.

Our own method of purifying casein appears to have been little used by others except at the University of Wisconsin. The procedure has been described in a previous paper.[5] It involves the handling of commercial casein in the following manner. The finely ground casein is placed in a tub and treated with a large amount of distilled water, to which acetic acid to the extent of about 0.2 per cent is added. About five pounds are handled at a time, and from eight to ten gallons of water are used. The first day the casein is washed by putting it on cheese cloth three times, and each time the greater part of the water is removed by moderate pressure on the cloth, after which the protein is returned to the tub and fresh water and acid are added. Thereafter for seven days more the change is made once a day. The repeated washing at first rapidly removes most of the sugar, soluble salts, and other contaminating substances on which bacteria would grow. The long continued washing with dilute acetic acid offers an opportunity for the soluble constituents to be extracted or to dialyse out of the granules. The acetic acid sets free the inorganic elements with which the acid groups of the casein molecule were combined, and these soluble acetates diffuse out of the granules and are washed away. Finally, the casein is soaked in water, drained, and then dried at about 75° C., and ground. This procedure results in a casein which is all but ash-free, and contains no demonstrable amounts of any vitamins. This method is, we are confident, much superior to that of extracting commercial casein with alcohol.

In selecting gelatin for experimental feeding, it is essential that only the highest grade be used, since the cheaper kinds contain, or are likely to contain, very considerable amounts of phosphorus and other inorganic elements. We have found Bacto-gelatin of the Digestive Ferments Company suitable, its only drawback being the high cost, which becomes a serious matter in extensive feeding studies. This we have analyzed and found to contain on an average about 0.120 per cent of phosphorus, and to be essentially free from other elements which might be of importance in influencing our results. Chemical analysis of gelatin should never be omitted in experimental work relating to rickets, and the small amounts of the significant elements which it furnishes should be taken into account in stating the content of any of these in the food mixtures.

3. Owing to the low ethical standards of certain manufacturers of inorganic salts which are widely used in chemical laboratories in this country, it is not safe to rely upon the label as evidence of chemical purity, or of the content of certain contaminating substances when these are stated, and purported to be based upon analysis by the manufacturer. There is much evidence to show that no labels are wasted, and that an analysis made at some time in the past may be still serving as a basis of guarantee today. Salts should be purchased in large quantities if possible and a chemical examination of each purchase should be made before use.

4. For the preparation of butter fat we have regularly placed about eight pounds of first class creamery butter in a double boiler and allowed it to melt as quickly as possible. The water, salts, casein, etc., separate and settle to form a lower layer, while the fat forms an upper layer. This is decanted off upon a large folded filter supported in a hot-water funnel, the water in which is maintained at boiling temperature. The filtered fat is collected in pint Mason jars which are closed, and are stored away from direct sunlight.

5. It should not be necessary to point out that extracts of wheat germ, now so widely employed as a source of water-soluble B, may likewise contain considerable amounts of fat-soluble A. It is not warranted to introduce liberal amounts of such extracts into diets used to study the fat-soluble A content of fats, for the results may be entirely vitiated by so doing. Thus, an alcoholic extract of ten grams of wheat germ per 100 grams of food may add a considerable amount of fat-soluble A to a diet in which lard or cottonseed oil are being studied, and erroneous conclusions may be drawn from the results of feeding the mixture.

## Discussion of Experimental Work

In a foot-note to Table I the quality of the basal food mixture which served in these experiments has been sufficiently discussed. It should be borne in mind, however, that although the diets employed in these studies are fairly complex in their composition, each component has been the object of careful special study in our earlier work, and that the specific dietary properties of each is well understood. It is possible to predict from these special studies the results which will be secured from feeding mixtures of natural foods, with or without supplementary additions of purified food substances.

Although the most significant features of the results of feeding the experimental diets described have been noted under remarks in Table I, there are certain points which need further consideration.

The diet of Lot 3143 gives exaggerated rickets when administered to young rats. The histological structure of the bones induced by this diet has been described in another paper.[5] We have made a special study of the extent to which slight modifications of this diet (our "line test" diet) interferes with the successful conduct of our "line test" for the calcium-depositing effect of any natural food. The following description of the histological characteristics of the bones of animals fed the series of diets described in Table I serves admirably to illustrate how very refined the experimental conditions must be maintained in order to avoid error in the conduct of this test.

If the diet of a young rat is modified so as to deviate from the composition of that of Lot 3143 in certain respects, distinct effects are observed in the histological picture of the bones (See Table I, Lot 3241). Under such conditions, even though the changes in the diet may

be of small magnitude, inconstancy of effects will be likely to occur and the data from a series of animals will appear confusing. To illustrate this the following modifications of diet 3143 will be described and their effects on the bones noted.

Diet 3241 produces changes which are identical with those seen in rachitic children. The epiphyseal disc of cartilage is much wider than normal and a rachitic metaphysis is formed between the cartilage and the shaft. The metaphysis is made up of a disorderly mass of osteoid tissue, connective tissue, blood vessels, and bone-marrow cells from the shaft of the bone. The trabeculæ of osteoid are separated from each other by dilated blood vessels and delicate reticular tissue, which may or may not contain a few marrow cells. Where cartilage cells are in contact with metaphyseal blood vessels, the cartilage may be in all stages of metaplasia into osteoid. Many of the cartilage cells degenerate. On the other hand, in places the cartilage shows a much greater tendency to retain its individuality and staining characteristics than it does in the bones of rats fed on diet 3143. Long tongues of cartilage persist in the metaphysis, which retain the morphology and staining characteristics of the parent tissue. In other words, the tendency of the cartilage cells to metaplasia and to degeneration is less pronounced than it is in the bones of animals on diet 3143. The metaphyses are not quite so wide, nor are the ends of the bones as greatly enlarged as those of animals on the latter diet. The bones of many of these animals show traces of incomplete healing. These take the form in sections of more or less complete or fragmentary lines of calcified intracellular substance crossing the metaphyses of the bones. This calcified intracellular substance results from the reformation of the zone of provisional calcification which is commonly the first sign of healing in the rachitic bone. When relapses occur after remissions, the metaphysis goes on growing and the new proliferative zone is not immediately resorbed, but remains buried in the new osteoid tissue. Some bones show traces of as many as three of these lines of calcified cartilage. Bones which show this picture are apparently identical with those human bones which show relapsing or healing rickets. The trabeculæ of the shaft are surrounded by broad zones of osteoid, but they are not more numerous than normal, and apparently little or no resorption of bone or osteoid occurs. The cortex is largely made up of osteoid tissue, and may be greatly thickened on one side. The marrow shows no change from normal, and there is no encroachment on the medullary cavity. The osteoid may or may not be lamellated, and the osteoid corpuscles are small.

These peculiarities of growth are due to the change in ratio between calcium and phosphorus in the diet. The ratio between these elements was nearer the optimum in this group (Lot 3241) than it was in that of Lot 3143, and the conditions were, therefore, more favorable to the production of normal bone, notwithstanding the shortage in the diet of the organic factor which favors the normal deposition of calcium. In Lot 3241, as in Lot 3143, the failure of the calcification was brought about by an excessive amount of calcium in the diet rather than by a deficit. The phosphorus content of both diets was essentially the same.

We may next consider the effects of reducing the calcium carbonate content of diet 3241 from 2 per cent to 1.5 per cent (See Table I, Lot 3228). This diet contained as nearly the optimal content of calcium as we can determine it at present. The phosphorus and the organic factor concerned in calcium deposition were contained in the diet in amounts almost exactly corresponding to those of Lots 3143 and 3241 discussed above.

The bones of animals on this diet in general differed only very slightly from normal. The cartilage was narrow and well calcified. The cortex and trabeculæ were slender, but were almost completely calcified. Only occasionally in the shaft was the border of osteoid tissue slightly wider than the physiological osteoid. There was some connective-tissue replacement of medullary tissue between the trabeculæ of the spongiosa, and a few large cells with basophilic granules were found in the immediate neighborhood of the trabeculæ. The cortex had a tendency to porosity in the neighborhood of the spongiosa. One of the animals on this diet showed the remains of calcified intercellular substance in the cortex as far down as the middle of the shaft of the bone. The trabeculæ were not as straight as those of normal bones, and the trabeculæ in the nucleus of ossification showed rims of osteoid tissue which were wider than those found in the normal animal. The bones of one animal, No. 1186, showed somewhat greater exaggeration of osteoid tissue than the rest of the series.

When we restricted young rats to diet 3227, which is the basal diet employed in this series, with no calcium additions, the bones were quite uniform in different animals.

This diet produced very imperfectly calcified bones. The cartilage in general was narrow and completely or nearly completely calcified. The trabeculæ were small, convoluted and very numerous, as they are in the bones which show osteosclerosis. The trabeculæ were incompletely calcified, and the calcium deposits in them had a granular appearance as though they were being rapidly disintegrated and rebuilt. They were partially or completely surrounded by zones of osteoid. A metaphysis was formed which had an orderly arrangement. The marrow was replaced between the trabeculæ by young connective tissue containing many basophilic cells. The osteoblasts varied greatly in size and shape. The majority had a deeply stained uniform cytoplasm. The bone corpuscles were often surrounded by a relatively clear area which was bordered by a granular deposit of calcium. There were abundant signs of resorption present in the spongiosa. The cortex of the shaft was a lattice-like condensation of spongiosa. In the nucleus of ossification signs of resorption were wanting, and the osteoid tissue was broader than the osteoid borders of the trabeculæ in the shaft.

The effect of modifying the ratio between calcium and phosphorus in our basal diet was further shown by certain experiments in which we have employed the same food mixture, Lot 3143 (see Table I), but with dicalcium phosphate added. This effect we may illustrate by Lots 3229 and 3173. Diet 3229 contained 2 per cent of calcium phosphate, which furnished the same amount of calcium as 1.5 per cent of calcium carbonate. Diet 3173 contained calcium phosphate equivalent to the calcium in 3 per cent of calcium carbonate.

The bones of animals in Lot 3229 were very well formed. The cartilage was somewhat narrow and was calcified throughout. The trabeculæ were not quite so straight as those in a normal bone, and tended to be thicker. They were completely calcified, had no osteoid tissue about them, and were surrounded by a layer of flat osteoblasts. The space between the trabeculæ was greater than normal and was filled with a delicate cellular connective tissue, which contained many basophilic cells and a few marrow elements. The cortex of the shaft was solid and very thick.

The center of ossification showed distinct porosis. The trabeculæ of the center were few, thick and completely calcified.

The bones in Lot 3173 differed very little from normal. Trabeculæ were more numerous and were straighter than in the bones of animals on diet 3229. Calcification was complete, save for the physiological osteoid tissue, which is evidence of normal growth.

We deemed it of importance to test the question whether several other calcium salts would yield comparable results in experiments of the character discussed. For this reason we tested the chloride and the lactate of calcium with our basal diet (See Table I). A rather surprising result was obtained with diet 3240, which contained calcium lactate 4.6 per cent (equivalent in its calcium content to calcium carbonate 1.5 per cent). Although this diet contained the same calcium: phosphate ratio as did diet 3228, the bones were not identical.

The bones of animals on diet 3240 were not abnormally thickened or deformed, but the histological picture was that of very mild rickets of low calcium type. The cartilage was abnormally broad, though the disc was not as wide as that which was produced by feeding diet 3142. It was incompletely calcified and had been invaded in places by blood vessels from the diaphysis, which had spared the calcified cartilage but invaded the rest of the disc. There was no definite wide rachitic metaphysis. The trabeculæ of the shaft abutted directly in many places on the cartilage. In this situation they consisted of little more than spicules of calcified intercellular matrix or bits of calcified cartilage surrounded by narrow borders of structureless osteoid. The latter might contain very many large-sized osteoblasts crowded together almost to the limit of the capacity of the osteoid; or the osteoid might contain almost no cellular elements. The large osteoblasts were oval or polyhedral and their cytoplasm was uniform in shape and was not so deeply stained. The osteoid corpuscles lay in lacunæ in the osteoid. In places the tissue in their neighborhood was infiltrated with sparse granular deposits of lime salts which did not enter the lacunæ or encroach on the cytoplasm of the cell. Even at the epiphyseo-diaphyseal border of these bones the trabeculæ showed granular deposits of calcium salts in their centers, which were the beginnings of ossification. The trabeculæ farther down in the shaft were largely made up of mature bone. The osteoid tissue in the growing region was either lamellated or structureless. It was covered in places with a single or multiple layer of very large osteoblasts, or it was quite bare, or surrounded partially or completely with flattened osteoblasts of an endothelioid form. The trabeculæ were separated by delicate reticular tissues, which supported numerous thin-walled blood vessels filled to capacity with blood cells. The connective tissues contained very few hæmatopoietic cells. A large number of osteoblasts lay free in it, and in some cases they were so numerous as to almost fill the space between the trabeculæ. The trabeculæ of the shaft were few in number. They were irregularly arranged and were surrounded by zones of lamellated osteoid tissue containing very few corpuscles. The osteoblasts which surrounded the osteoid in this region were of a flattened endothelioid type. The cortex was merely a lattice-like condensation of the spongiosa. The histology of the center of ossification corresponded to that of the shaft. In the growing region of the bone there were many signs of resorption as well as of active bone formation. Osteoblasts were fairly numerous but were not present in extraordinary numbers. The newly formed trabeculæ showed many signs which can only be interpreted as evidence of bone destruction. Large numbers of perforating blood vessels were to be seen in these trabeculæ in many places. In others the osteoid tissue and the bone were being eroded by vascular loops from the diaphyseal circulatory system. In still other places newly built bone and calcified cartilage, partly stripped of its osteoid, seemed to be crumbling away under the influence of cellular and vascular activity. Here and there, large cells filled with basophilic granulations were to be found in approximation to the trabeculæ. They were most numerous in the cortex and the older parts of the spongiosa. The bone marrow was apparently normal.

It will be seen that, in spite of the fact that the ratio between calcium and phosphorus was precisely the same in diet 3240 as in diet 3228, which with one exception produced animals with approximately normal bones, the bones of animals on diet 3240 were affected with a mild grade of atypical rickets. Moreover, the histology of the lesion would suggest that this rickets was a mild grade of the low calcium type. The sole difference between the two rations was that the calcium in diet 3240 was supplied in the form of the lactate, whereas that in diet 3228 was in the form of the carbonate. It is not possible at present to offer a satisfactory explanation for this difference in the relative effectiveness of the carbonate and lactate of calcium in promoting the development of the bones.

Lot 3236 contained 9.2 per cent of calcium lactate. This amount was sufficient to make the calcium content of the food equal to that of Lot 3143, which had 3 per cent of added calcium carbonate.

The bones of these animals were affected with very severe rickets, which corresponded exactly with the severest type commonly seen in children. At the same time, although the calcium-phosphate ratio in this diet was exactly the same as that in diet 3143, the bones bore a closer resemblance to those which resulted from the administration of diet 3241 (which contained 2.0 per cent of calcium carbonate) than to those which were produced as the result of feeding the former diet. The bones of animals of lots 3236 and 3241 differed from those of 3143 in that there was a greater tendency for the cartilage cell to retain its individuality and primitive characteristics in the former two. Moreover, on diet 3236, as on diet 3241, abortive attempts at healing with reformation of the provisional zone of calcification occurred, which left traces in the form of more or less complete lines of calcified intercellular substance in the cartilage and metaphysis of the bone.

Lot 3235 received calcium chloride 3.4 per cent (equivalent in calcium content to 3.0 per cent calcium carbonate). Animals on this diet developed very severe rickets.

The pathological picture of these bones was comparable to that seen in rats on diet 3143, except that the epiphyseal disc was slightly wider than that found in the bones of animals on the latter formula. There was a slightly greater tendency on the part of the cartilage cells to maintain their individuality and staining characteristics on diet 3235 than on 3143. Therefore, since the epiphyseal disc was invaded by numerous capillaries from the shaft, the epiphyseal disc had a very ragged margin on the metaphyseal side. Although the metaphysis of these bones was wider than the metaphysis of bones of animals on diet 3241, they were alike in that traces of abortive attempts at healing were found in the metaphysis. These were in the form of broken lines of calcified intercellular substance which were embedded in the osteoid of the metaphysis at right angles to the long axis of the bones.

TABLE SHOWING COMPOSITION OF DIETS AND THE NATURE OF THEIR DEFICIENCIES

| No. of diet | Wheat | Maize | Gelatin | Wheat gluten | NaCl | $CaCO_3$ | $CaCl_2$ | Calcium lactate | $CaHPO_4$ | Ca in food | P in food | Wt. ratio Ca : P | Atomic ratio Ca : P | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gms. | Gms. | Gms. | Gms. | Gms. | Gms. | Gms. | Gms. | Gms. | Gms. | Gms. | | | |
| 3143 | 33 | 33 | 15 | 15 | 1 | 3 | | | | 1.2316 | 0.3019 | 1:0.2531 | 1:0.3163 | This diet produces a very exaggerated form of rickets. |
| 3241 | 34 | 33 | 15 | 15 | 1 | 2 | | | | 0.8320 | 0.3059 | 1:0.367 | 1:0.472 | This diet produces bones which are identical with those seen in rachitic children. |
| 3228 | 43.5 | 33 | 15 | 15 | 1 | 1.5‡ | | | | 0.6320 | 0.3059 | 1:0.4840 | 1:0.6245 | These bones in general differ only slightly from normal. |
| 3227 | 36 | 33 | 15 | 15 | 1 | | | | | 0.0322 | 0.3101 | 1:9.630 | 1:12.430 | These bones would seem to indicate that in this ration the phosphate tended to over-balance the calcium in its effects. |
| 3229 | 34 | 33 | 15 | 15 | 1 | | | | 2* | 0.497 | 0.6668 | 1:1.3416 | 1:1.298 | Bones well formed. Trabeculæ not so straight as those of normal bone. |
| 3173 | 33 | 32 | 15 | 15 | 1 | | | | 4** | 0.962 | 1.0277 | 1:1.0683 | 1:1.3718 | Bones of these animals differ very little from the normal. |
| 3240 | 31.4 | 33 | 15 | 15 | 1 | | | 4.6† | | 0.6303 | 0.2938 | 1:0.4661 | 1:0.6015 | Histological picture is that of mild rickets of the low calcium type. |
| 3236 | 26.8 | 33 | 15 | 15 | 1 | | | 9.2†† | | 1.2305 | 0.2700 | 1:0.2194 | 1:0.2831 | Very severe rickets corresponding to human type. |
| 3235 | 32.6 | 33 | 15 | 15 | 1 | | 3.4 | | | 1.2303 | 0.3019 | 1:0.2454 | 1:0.3166 | Very severe rickets. |

* Furnished calcium equivalent to that in 1.5 grams $CaCO_3$, and phosphorus 0.3609 grams.
** Furnished calcium equivalent to that in 3.0 grams $CaCO_3$, and phosphorus 0.7218 grams.
† Furnished calcium equivalent to that in 1.5 grams $CaCO_3$.
†† Furnished calcium equivalent to that in 3.0 grams $CaCO_3$.
‡ Contained 0.600 grams of calcium.

The quality of all the diets listed in the table was essentially identical as respected protein, fat soluble A, water-soluble B, and the uncharacterized substance which plays a rôle in directing the growth processes in the bones. None of these diets were capable of inducing growth at a rate faster than half the optimal for a period of a few weeks. This was due to deficiency of fat-soluble A, and except in the cases of diets 3229 and 3173 to lack of sufficient phosphorus. When these faults are corrected these food mixtures are all capable of inducing good nutrition in rats through successive generations.

We could not detect any influence of the chloride, which contains a non-oxidizable acid radical, in contrast with the carbonate or lactate, which are both potential neutralizers of acid in the body.

The results reported in this paper serve to extend and confirm our previous studies on the effect of deviating from the optimal ratios between calcium and phosphorus in the diet when the organic factor is low. They leave no room for doubt as to the importance of the relation between these two elements. The primary purpose in presenting these data was, however, to emphasize how one may readily fall into error in studying the anti-rachitic effect of any substance, unless the greatest care is taken to fulfill the conditions which we have so carefully standardized.

## CONCLUSIONS

Dietary conditions are discussed which must be fulfilled in conducting our "line test" for the calcium-depositing substance (anti-rachitic effect) of foodstuffs.

The importance of selecting the highest quality of food grains; of using a rational system of protein purification; the use of salts of proven quality, and of taking into account the content of fat-soluble A in alcoholic extracts of wheat germ or other substance which may be employed as sources of water-soluble B, are discussed and emphasized.

The pronounced anatomic changes which are brought about by relatively small variations in the composition of the diet, with respect to those factors which play a rôle in bone growth, are illustrated by specific examples from our experimental data.

The lactate of calcium proved a less satisfactory source of this element for promoting growth of the bones than did the carbonate. The cause of this difference must await further investigation.

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# NOTES ON NEW BOOKS

*Bibliography of the Writings of Sir William Osler, Bart., M.D., F.R.S. Regius Professor of Medicine in the University of Oxford.* By MINNIE WRIGHT BLOGG, librarian of the Johns Hopkins Hospital.

This book is an amplification and virtual perfection of the bibliography of the writings of the late Dr. Osler, which appeared in the Johns Hopkins Hospital Bulletin in 1918. The original publication presented 730 titles, the number being increased later to 773 by titles subsequently sent by the author from England. The indefatigable compiler of the present volume, Miss Blogg, has now brought it up to 1195. The volume before us must, therefore, be considered as the definitive record of the writings of this remarkable man. The task has been an immense one and the thanks of medical men are due to her for her arduous labor. The varied character of Osler's writings is here characteristically shown. There are contributions on physiology, pathology and practical medicine, medical biography, the history of medicine, and reports of hospital practice and general medicine. There are also numerous text-books, the editions of which he so conscientiously revised for their progressive improvement, and monographs upon special subjects. Not the least interesting portion of the titles are the communications, notes and even editorials, which he supplied to the medical journals in which he was interested while a resident of Montreal, Philadelphia, Baltimore and Oxford. The wonderful industry and versatility of Dr. Osler is shown in the many additions which have been made to the original edition of this book. He was interested in every phase of medical knowledge and equally interested in all that pertained to medical education, and the contact between medicine and social service. The present edition of his bibliography is a monument also to the industry and painstaking work of the compiler. It should be on the desk of everyone interested in medical bibliography.

H. M. H.

*The Diseases of Infants and Children.* By J. P. CROZER GRIFFITH. (*W. B. Saunders Company, Philadelphia and London, 1919.*)

In presenting these two volumes the author has attempted to bridge the gap between the smaller text-books and a complete system of Pediatrics. The volumes are well edited and contain a moderate number of good illustrations. The author has drawn on his wide clinical experience and in addition shows a comprehensive knowledge of the native and foreign literature. The reader will profit by the numerous references which he gives and it is pleasing to note that he has referred to so many of the original articles on different subjects.

In discussing artificial feeding in infancy the author rightly emphasizes the point that there must be no fixed method of feeding. He shows that the "percentage method" and the "caloric method" of feeding are merely the outcome of certain basic principles of nutrition. The two methods are really complementary and not diametrically opposed to one another, as has so often been imagined.

Except for two short chapters on infantile atrophy and malnutrition, the nutritional disorders of infancy are discussed primarily as diseases of the digestive system. Although it is true that many of the symptoms are referable to the gastro-intestinal tract, the primary cause of most of these disorders is not a local lesion or functional disturbance but rather a disturbed tolerance of the whole body for certain food stuffs. Such conditions arise from many causes, as, for example, an improper balance in the food elements, overfeeding, and parenteral infection. In the last named the digestive disturbances are merely secondary complications. As a matter of fact we often see a profound nutritional disorder from improper feeding, with few or no digestive symptoms. If progress is to be made in this important subject a broader view-point than the author's must be adopted.

Apart from this subject the book is well handled. The chapters on systemic diseases are good and due consideration is paid to their peculiar characteristics in childhood. We feel that rheumatic fever might be considered under the infectious diseases, for although its etiology is still in doubt, it is certainly an infectious process. Bacillary dysentery should also be included under the same heading. Dr. Griffith doubts the value of the agglutination reaction in dysentery, although the majority of workers consider that this test, if properly done, gives a specific reaction in children, showing the presence or absence of infection with the dysentery bacillus.

One must take exception to the statement that *B. coli* is unable to grow in an alkaline medium. This has been shown to be incorrect. In his discussion of tetany the author writes: "The outcome is never fatal, although death may occur depending upon the primary disease or upon complications." However, he says that death may occur during attacks of laryngospasm occurring in this disease. As this is an integral part of the disease, we feel that his statement may give rise to misconceptions.

For students and practitioners the book may be recommended; in conjunction with more complete works on special subjects it will fill a place in the library of a specialist.

S. G. R.

*The Evolution of Modern Medicine.* By SIR WILLIAM OSLER. (*London, New Haven, Oxford University Press, Yale University Press, 1921.*)

Throughout the writings of Sir William Osler may be discerned an eager interest in the historical and bibliographic side of medicine. Steeped as he was in the classics and in their modern reincarnations, and endowed with the deepest instincts of the true literateur, it seemed all but impossible for him to write without apt allusion or allegory derived from

older sources. Among his works we find no less than 61 titles which may be said to deal purely with historical matters. It is altogether befitting, therefore, that we should have left to us one larger and reflective piece of historical writing from his pen.

"An airplane flight through the centuries" he calls it—and rightly—for men and events flash by; but there is no blurring, and when we close the volume the whole story is sharply scored on the tables of the mind. The idea of the origin of medicine from vague religious and social impulses is developed, then the first definite crystallization of the art with Asklepios and Hippocrates, and later its expansion under Galen, the dark days of the crash of the Western Empire, the lucky rescue of medicine from oblivion by the curious and indirect route of Arabia, while mediæval mysticism held sway in Europe, the Renascence, and finally the rapid and inevitable developments of the modern era. But there is much more than the story of events. The story of ideas is developed with equal keenness. Osler has written not merely a chronicle but a piece of historical reflection of the very highest order.

Few readers in this day and age will read between the lines and grasp the real meaning of the book. It expresses Osler's creed—Grecian from start to finish—more directly stated in "The Old Humanities and the New Science." He realized well that despite material advance in science the road may lead not into Attica but to Bœotia. Never a reformer, he influenced men by precept and by example, and this History more than anything he has done stands for the broad and generous point of view in medicine.

A. L. B.

*Acute Epidemic Encephalitis (Lethargic Encephalitis).* An Investigation by the Association for Research in Nervous and Mental Diseases. (*New York; Paul B. Hoeber, 1921.*) $2.50.

Despite the great importance of epidemic encephalitis the disease has not become common enough for any single observer's experience to be altogether comprehensive and authoritative. One welcomes, therefore, this collective investigation by such men as Barker, Hunt, Amoss, and Griffith, to mention only a few of the names of those concerned. The material of the book includes sound and critical summaries of the various facts which have been collected about encephalitis, each man presenting as it were "the evidence" in his domain of special interest. There is some question in the reviewer's mind as to whether the value of the book is enhanced by the verbal discussions at the end of each section which are reported verbatim apparently from extemporaneous remarks, and which perhaps carry the analogy to court proceedings a little too far. For the time being, however, this book must be regarded as a most valuable storehouse of information about an obscure and difficult disease.

A. L. B.

*Oxford Medicine, Vol. V.*, Edited by HENRY A. CHRISTIAN and SIR JAMES MACKENZIE. (*New York and London; Oxford University Press, 1922.*)

Volume V. of the Oxford Medicine continues along the general lines of its predecessors. Interesting summaries of the infectious diseases are presented from a modern and satisfactory point of view.

A. L. B.

*The Vitamins*, by H. C. SHERMAN and S. L. SMITH. American Chemical Society Monograph Series. (*New York, Chemical Catalogue Company, 1922.*)

At a time when the lay mind is being actively stimulated to an interest in "vitamins," both by popular writers and manufacturers of various commercial vitamin products, it is very desirable to have a review of the actual scientific knowledge of these substances, particularly of the sources from which they are readily and easily obtainable. To the physician, who is frequently besieged by questions on this subject, the concluding statement of the book is significant:—"We believe it is safe to say that with a dietary selected to make the best use of our ordinary staple foods there will rarely, if ever, be occasion to purchase vitamins in any other form, or to give any greater anxiety to the vitamins than to some other factors which enter into our present conception of nutritive requirements and food values." In the concluding chapter there is a useful table showing the distribution of "vitamins" in investigated food materials.

The bibliography comprises about one thousand titles. It is remarkable and very significant that the authors have been able to condense such an enormous literature into such a small space. To one who has watched the rapidity with which publications in this field have issued from the various laboratories, filled with hastily drawn conclusions and polemics, the task of making such a review is appalling. The authors have handled this difficult task without the introduction of personal bias. One feels that in some respects the review is not sufficiently critical.

It is of value for reference because of its unbiased point of view, and because it assigns to "the vitamins" no more than their proper share of importance in the broad field of the science of nutrition.

W. S. McC.

*The Clinical Study of the Early Symptoms and Treatment of Circulatory Disease in General Practice.* By R. M. WILSON. (*Oxford Medical Publications, London, 1921.*)

> "You might just as well say, added the Dormouse, .... that I breathe when I sleep is the same thing as I sleep when I breathe."
>
> ALICE IN WONDERLAND.

To those readers at all familiar with the author's views as expressed in his earlier work entitled *The Hearts of Man* many of the ideas set forth in this volume will come as no surprise, but to the uninitiated there will be much, we fear, that appears obscure in the elucidation of his unique hypothesis.

We confess at the outset that it was not without great effort that we were able to follow the author's frequently confused text and that our interest flagged tremendously long before the end came in sight. It would be equally false if we conveyed the impression that there are not a number of very sound truths contained in this volume. Unfortunately, many of them are lost in a bewildering maze of paradoxes that are indeed difficult to unravel and too often destructive of the author's purpose.

The raison d'être of the work is to set forth in great detail the author's theories of what we may call the balanced action met with in every physiological reaction, whether normal or pathological, and an attempt to analyze the effect of a stimulus, and the reaction following, upon the insecure basis of clinical observations alone.

A clue to the author's general attitude toward established physiological truths is offered early in his introduction when he says: "On the experimental table stimulation of the vagus slows the heart. But in clinical practice methods employed to this end *always* (italics ours) result in quickening it, even if a temporary slowing may at first occur!" The first five chapters devoted to the subject of Exhaustion constitute an attempt to apply the author's theories to the elucidation of a very complex problem due chiefly, he asserts, to the influence of pathological stimuli affecting primarily either the vagus or the sympathetic nerves. He postulates an extraordinary, one might say speaking paradoxically, almost human relationship between the vagus and sympathetic fibres frequently described as follows: "Thus to

borrow a phrase from the commercial world the cheques drawn by the vagus in its responses to stimuli are honored by the sympathetic." On page 39 he says: "Exhaustion, then, in a toxic state is first of all a matter of vagus excitability." A little further on "The toxic patient, then, presents a vagal picture (exhaustion) when and because reciprocal action by the sympathetic fails owing to depletion of that system. The patient with a mechanical disturbance in the viscera is in a different plight. The visceral disturbance causes sympathetic stimulation and so reflex vagal activity, and the latter soon dominates the former just as in response to outside stimuli the sympathetic when healthy soon dominates the vagus."

The chapter devoted to a study of Breathlessness is based upon a further application of the theory of vagal and sympathetic interplay. He divides breathlessness into two main groups, the "reaction type" of toxic origin due to action on the vagus, the "resting type" due to sympathetic exhaustion which in time may give place to a so-called "diaphragmatic type," when the respiratory rate increases and death is at hand. There is no discussion of the physiology of cardiac dyspnœa, although allusion is made to the work of Haldane and Meakins and there is an occasional reference to oxygen want and oxygenation and the importance of the circulation.

The short section dealing with cyanosis is utterly unsatisfactory. All critical discussion of or even reference to the vast amount of work in recent years bearing on the subject is omitted. Referring to the incidence of cyanosis in pneumonia, its occurrence at the time of onset is over-emphasized and explained on the basis of an "invasion in mass of the toxin producing a very great vagus excitability to which the sympathetic could not at once respond."

The remaining chapters of the book are concerned with such topics as the symptoms of the patient's sensations, dealing with hyperalgesia, cardiac pain and headache, observations on blood pressure, the earliest symptoms of heart failure, and concluding with suggested treatment based upon early signs and symptoms; the whole discussed on the basis of the author's theory of the fundamental importance of the nervous system in relation to the subjective and objective evidences of cardiac disease.

The volume is introduced by a foreword from the pen of Sir James Mackenzie, in which he says that "Whether, then, the hypothesis which Dr. Wilson puts forward.....is confirmed or disproved, he has rendered a service to medicine. Those who may cavil at such a work must bear in mind that here is but the beginning of a big undertaking." It is indeed difficult to understand the point of view which seems to have overtaken this distinguished observer in recent years. It is quite true, we admit, that in clinical medicine two of the most important considerations "are the prognostic significance of a symptom and the mechanism of its production," but above all we need carefully controlled and interpreted observation. We cannot describe in too accurate detail our facts, but we should ever be cautious and over-critical in our conclusion based upon unsupported evidence.

E. P. C.

*A Manual of Selected Biochemical Methods as Applied to Urine, Blood and Gastric Analysis.* By FRANK P. UNDERHILL, XIV+ 232 pp. (*New York: John Wiley & Sons; London: Chapman & Hall, Ltd., 1921.*)

It is a distressing fact that biochemical methods of analysis are constantly changing and multiplying at a prodigious rate, and that manuals of such methods are short-lived undertakings. Yet Professor Underhill's compact volume presents to the biochemical analyst a more valuable and useful compendium than he has had hitherto. The great variety of the older and more modern methods of urine, blood, and gastric analysis are included, covering more than the ordinary routine procedures of a clinical laboratory, and furnishing ample substance for research work along these lines. A number of new and useful items for a manual of this kind are introduced, such as calibration of apparatus, acidimetry and alkalimetry, standardization of oxidizing solutions, etc. From the standpoint of instruction, the excellent plan of giving the principle involved in every analytical procedure has been faithfully followed. The different methods are mostly quoted verbatim from the original sources, perhaps too faithfully, with little attempt to clarify directions which are not clear or explicit enough in places. No critical appraisal is presented by the compiler on the comparative accuracy or simplicity of the methods, with the obvious presumption that each worker will choose the particular method which happens to suit his needs best. References being given in every instance to the original article, the reader is incited to study the method in which he is interested at greater length. The lack of tables comparing the content of urine and blood in normal and various pathological constituents as approximate guides in interpreting analytical results is also felt. Neither does the reader find any discussion of such debatable problems as the preferability of the use of whole blood or of plasma for the analysis of various constituents. It is hoped that this excellent manual will be revised from time to time so as to include the more valuable of the accretions to the biochemical methodology, and that it will have the wide use which it easily merits.

W. A. P.

# BOOKS RECEIVED

*A Psychiatric Milestone.* Bloomingdale Hospital Centenary 1821-1921. 8°. 220 pages. Privately Printed by the Society of the New York Hospital. 1921.

*Arabian Medicine.* Being the Fitzpatrick Lectures Delivered at the College of Physicians in November 1919 and November 1920. By Edward G. Browne, M.B., F.R.C.P., 1921. 8°. 138 pages. University Press, Cambridge.

*The Harvey Lectures.* Delivered Under the Auspices of the Harvey Society of New York, 1919-1920. By Lieut. Colonel George Dreyer, Dr. H. H. Dale, and others. Series XV. 1921. 8°. 157 pages. J. B. Lippincott Company, Philadelphia and London.

*Nostrums and Quackery.* Articles on the Nostrum Evil, Quackery and Allied Matters Affecting the Public Health; Reprinted, With or Without Modifications, from the Journal of the American Medical Association. Prepared, Compiled or Edited by Arthur J. Cramp, M.D. Volume II. 1921. 8°. 832 pages. Press of the American Medical Association, Chicago.

*Menstruation and Its Disorders.* By Emil Novak, A.B., M.D., F.A.C.S. With Forty Illustrations. Gynecological and Obstetrical Monographs. 1921. 8°. 357 pages. D. Appleton and Company, New York and London.

*Episcopal Hospital,* Medical and Surgical Reports. Volume V, 1917-20. 8°. 506 pages. Wm. J. Dorman, Philadelphia.

*The Submucous Resection of the Nasal Septum.* By W. Meddaugh Dunning, M.D. 1921. 12°. 97 pages. Surgery Publishing Company, New York.

*Studies in Influenza and Its Pulmonary Complications.* By D. Barty King, O.B.E., M.A., M.D. (Edin.), M.R.C.P. (Lond. and Edin.). With 31 illustrations. 1922. 8°. 88 pages. J. & A. Churchill, London.

*The Vitamins.* By H. C. Sherman and S. L. Smith. American Chemical Society, Monograph Series. 1922. 8°. 273 pages. The Chemical Catalog Company, Inc. New York.

*The Treatment of Common Female Ailments.* By Frederick John McCann, M.D., Edin., M.R.C.P., London, F.R.C.S., England. 1922. 8°. 152 pages. Edward Arnold & Co., London.

*Vice and Health Problems—Solutions.* By John Clarence Funk, M.A., LL.B. 1921. 12°. 174 pages. J. B. Lippincott Company, Philadelphia and London.

*Ephraim McDowell "Father of Ovariotomy" and Founder of Abdominal Surgery.* With an Appendix on Jane Todd Crawford. By August Schachner, M.D., F.A.C.S. 1921. 8°. 331 pages. J. B. Lippincott Company, Philadelphia and London.

*Acute Epidemic Encephalitis (Lethargic Encephalitis).* An Investigation by The Association for Research in Nervous and Mental Diseases. Report of the Papers and Discussions at the Meeting of the Association; New York City, December 28th and 29th, 1920. Prepared under the direction of Walter Timme, M.D., Pearce Bailey, Lewellys F. Barker, Sanger Brown, and others. 1921. 12°. 258 pages. Paul B. Hoeber, New York.

*The Evolution of Modern Medicine.* A Series of Lectures Delivered at Yale University on the Silliman Foundation in April, 1913. By Sir William Osler, Bart., M.D., F.R.S. 1921. 4°. 243 pages. Yale University Press, New Haven. Humphrey Milford, Oxford University Press, London.

*Clinical Diagnosis.* A Text-Book of Clinical Microscopy and Clinical Chemistry for Medical Students, Laboratory Workers, and Practitioners of Medicine. By Charles Phillips Emerson, A.B., M.D. Fifth edition entirely rewritten and reset. 156 illustrations. 1921. 8°. 276 pages. J. B. Lippincott Company, Philadelphia and London.

*An Introduction to Biophysics.* By David Burns, M.A., D.Sc. With a foreword by D. Noel Paton, M.D., LL.D., F.R.S. With eighty-five illustrations. 1921. 8°. 435 pages. Macmillan Company, New York.

*A Form of Record for Hospital Social Work:* Including Suggestions on Organization. By Gertrude L. Farmer. 1921. 8°. 81 pages. J. B. Lippincott Company, Philadelphia, London and Montreal.

*Epidemiology and Public Health.* A Text and Reference Book for Physicians, Medical Students and Health Workers. In Three Volumes. By Victor C. Vaughan, M.D., LL.D. Assisted by Henry F. Vaughan, M.S., D.P.H., and George T. Palmer, M.S., D.P.H. Vol. I. Respiratory Infections. 1922. 8°. 688 pages. C. V. Mosby Company, St. Louis.

*Book on the Physician Himself.* From Graduation to Old Age. By D. W. Cathell, M.D. This is the Vastly Improved Crowning Edition. 1922. 8°. 359 pages. Published by the Author, Baltimore, Maryland.

*The Mechanics of the Digestive Tract.* By Walter C. Alvarez, M.D. With twenty-two illustrations. 1922. 8°. 192 pages. Paul B. Hoeber, New York.

*Neoplastic Diseases;* A Treatise on Tumors. By James Ewing, A.M., M.D., Sc.D. Second edition, revised and enlarged with 514 illustrations. 1922. 8°. 1054 pages. W. B. Saunders Company, Philadelphia and London.

*Clinical Electrocardiography.* By Fredrick A. Willius, B. S., M.D., M.S. in Medicine. With 185 illustrations. 1922. W. B. Saunders Company, Philadelphia and London.

*University of Minnesota.* Papers from the Mayo Foundation for Medical Education and Research and the Medical School. Volume I, 1915-1920. 1921. 8°. 659 pages. W. B. Saunders Company, Philadelphia and London.

*An Introduction to the Study of the Protozoa:* With Special Reference to the Parasitic Forms. By E. A. Minchin, M.A., Ph.D., F.R.S. Second impression, illustrated. 1922. 8°. 517 pages. Edward Arnold, London.

*Diseases of the Eye.* A Handbook of Ophthalmic Practice. By George E. de Schweinitz, M.D., LL.D. (Univ. of Pa.). Ninth edition, reset, with 415 illustrations and 7 colored plates. 1922. 8°. 832 pages. W. B. Saunders Company, Philadelphia and London.

*An Essay on the Physiology of Mind.* An Interpretation Based on Biological, Morphological, Physical and Chemical Considerations. By Francis X. Dercum, A.M., M.D., Ph.D. 1922. 12°. 150 pages. W. B. Saunders Company, Philadelphia and London.

*Abdominal Pain.* By Prof. Dr. Norbert Ortner. Authorized Translation by William A. Brams, M.D., and Dr. Alfred P. Luger. 1922. 12°. 362 pages. Rebman Company, New York.

*Protein Therapy and Nonspecific Resistance.* By William F. Petersen, M.D. With an introduction by Joseph L. Miller, M.D. 1922. 8°. 314 pages. Macmillan Company, New York.

*Clinical Tuberculosis.* By Francis Marion Pottenger, A.M., M.D., LL.D. With a Chapter on Laboratory Methods, by Joseph Elbert Pottenger, A.B., M.D. Vol. I. *Pathological Anatomy, Pathological Physiology, Diagnosis, and Prognosis.* Vol. II. *Complications and Treatment.* Second edition. 1922. 8°. C. V. Mosby Company, St. Louis.

*Oxford Medical Publications.* Publishers: Henry Frowde, London; Hodder & Stoughton, London. The following five volumes:

*Arterial Sclerosis.* A Consideration of the Prolongation of Life and Efficiency After Forty. By Louis Faugeres Bishop, M.A., M.D., Sc.D., F.A.C.P. 1921. 8°. 383 pages.

*Hygiene of Women and Children.* By Janet E. Lane-Claypon, M.D., D.Sc. (Lond.). 1921. 8°. 354 pages.

*Bowel Diseases in the Tropics. Cholera, Dysenteries, Liver Abscess and Sprue.* By Sir Leonard Rogers, C.I.E., M.D., F.R.C.P., F.R.C.S., I.M.S. (Retired). 1921. 8°. 475 pages.

*The Clinical Study of the Early Symptoms and Treatment of Circulatory Disease in General Practice.* By R. M. Wilson, M.B., Ch.B. With a foreword by Sir James Mackenzie, M.D., F.R.S., F.R.C.P. 1921. 8°. 245 pages.

*Medical Electricity for Students.* By A. R. L. Browne. 1921. 12°. 231 pages.

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